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Clinical chemistry in the high school classroom

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CLINICAL CHEMISTRY
IN THE
HIGH SCHOOL CLASSROOM

A Thesis

Presented to

The Faculty of the Department of Chemistry

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ABSTRACT

CLINICAL CHEMISTRY IN THE HIGH SCHOOL CLASSROOM

by Jennifer A. Gray

The purpose of this thesis is to develop clinical chemistry modules for the Advanced Placement High School chemistry curriculum. Clinical Chemistry is defined as the chemistry of human health and disease management. This subject is an important area of science that integrates chemistry, biology, and physiology. It demonstrates the relationship of chemistry to the molecular processes within the body. This thesis project contains an educational review of teaching and learning theory as it applies to high school chemistry, a review of clinical chemistry, and five written lesson plans in clinical chemistry.

Each of the five lesson plans has a set of objectives that includes "hands-on" clinical diagnostic tests. These "hands-on" tests along with diagrams and demonstrations provide students with a concrete cognitive level learning task as defined by Piaget. Students will extend their knowledge further by correlating specific disease states to the test results, which is the highest level of Piaget's cognitive skills.

Students will learn about "body chemistry" and how to perform clinical tests that facilitate health management.

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INTRODUCTION

The purpose of this thesis is to develop clinical chemistry modules for the Advanced Placement (AP) high school chemistry curriculum. Clinical chemistry is defined as the chemistry of human health and disease in the management of patients (Stedman's Medical Dictionary, 1990). This thesis contains an educational review of teaching and learning theory as it applies to high school chemistry, a review of clinical chemistry, and five written lesson plans in clinical chemistry.

The educational review describes Piaget's model of the stages in developing cognitive abilities. It is a compilation of studies conducted on high school and college level students based on their abilities to learn specific concrete and formal reasoning skills in chemistry. Research shows that over 50% of the high school students perform at a concrete reasoning level (Jackson, 1965). Therefore, a majority of high school students need to be more active and involved in "hands-on" activities to develop formal reasoning skills.

The chemical literature review is composed of five sections and reflects the curricular content of these lesson plans. The five sections are: (1) Introduction to the Clinical Laboratory, (2) Diagnosing Glucose Metabolites in the Blood and Urine, (3) The Blood Gases, (4) Analysis of Analytes and Cells in the Urine, and (5) Antigen and Antibody Reactions. Each of these topics contains clinical tests that are performed for the purpose of diagnosing certain diseases. These five areas are chosen because the topics are relatively simple and they can be applied to humans. For comprehension at the concrete level, each topic relates to health scenarios for which students can apply clinical tests. A medical glossary is found at the end of the thesis to define the medically-related words.

Each of these lesson plans has specific objectives geared for students' understanding of clinical chemistry. The objectives are taught using different learning techniques such as cooperative learning, acting, writing exercises, and laboratory tests. *Introduction to the Clinical Laboratory*, the first lesson plan, focuses on introducing clinical chemistry concepts as well as units of measurement in the clinical laboratory. The students will become familiar with the meaning and interpretation of clinical laboratory results by completing Patient History Charts with their classmates. Moreover, they will learn about quality control, safety, and analytical methods in the clinical laboratory. The students will gather in cooperative groups to learn the different stages of disease (ie., diagnosis, prognosis, monitoring, and screening) and act out their own stage of disease in front of the class to see if the class can learn the stages of disease visually. For example, two students may choose to act out the stages of disease where one student plays the doctor and one student plays the patient.

In this lesson plan, a review of the digestions of proteins, fats, and carbohydrates will also be completed using cooperative groups. By using this technique, the students will actively work together to study the digestion of proteins, fats, and carbohydrates.

The second lesson plan, *Diagnosing Glucose Metabolites in the Blood and Urine* concentrates on the metabolism of glucose levels in the blood. Students will perform tests to determine the glucose and ketone body concentration in the urine. Then, they will correlate these glucose and ketone body levels to diseases related to glucose metabolism such as diabetes mellitus. Students will study case history tests of patients with glucose-related diseases and will learn to use different clinical tests depending on the disease. Furthermore, they will research a disease related to glucose metabolism in the library and do an oral group presentation in front of the class.

The third lesson plan, *The Blood Gases*, focuses on the concept of equilibria in chemistry. Since the Advanced Placement students have already learned the definition of buffers, this lesson is designed to use one specific buffer, the carbonic acid-bicarbonate pair to study blood gas equilibria. When the blood gases are not in equilibrium, certain underlying symptoms form depending on the pH of the blood. The students will complete exercises to observe the physiological changes in their bodies and apply these exercises to the carbonic acid-bicarbonate buffer. They will analyze gamblegrams, which are blood gas charts that show the different levels of the blood gases in the artery. With these charts, the students can conclude if the patient has respiratory acidosis, respiratory alkalosis, metabolic acidosis, or metabolic alkalosis.

The fourth lesson plan, *Analysis of Analytes and Cells in the Urine*, is concerned with the analytes found in the urine. The students will evaluate their own urine and analyze diseased urine, where the teacher will add chemicals to illustrate a "hypothetical" patient's urine. They will test for proteins, ketone bodies, glucose, pH, specific gravity, nitrite, and leukocyte esterase. In addition to the qualitative tests, the color of the urine and odor of the urine will be observed. The students will be able to correlate the various levels of these analytes to diseases such as diabetes mellitus and proteinuria. Moreover, the students will do a microexamination of the urine to observe the normal cells found in the urine, such as squamous and epithelial cells. They will look at abnormal urine specimens that contain white blood cells, red blood cells, crystals, and yeast cells. The students will be given an unknown specimen to evaluate and, to diagnose the disease(s) present in this urine specimen.

The last lesson plan, *Antigen and Antibody Reactions*, contains a unit on the interaction of antigens and antibodies. Students will perform an Ouchterlony Test using bovine, goat, and sheep sera to learn the specificity of antigen and antibody reactions.

Since the State of California does not permit blood to be used in the high school classroom, A, B, O blood typing will be demonstrated by the instructor. Although the students cannot handle blood, they can determine whether they secrete their blood antigens or not. If students note the bitter taste of phenolthiacabamazine, they can conclude that they secrete their blood antigens.

These lesson plans are used to encourage "hands-on" activities for the students, so they can apply these concrete concepts one step further to more formal concepts. It is anticipated that these lesson plans will be used as a pilot program to determine the outcome of the clinical chemistry curricula devised in this thesis.

The five lesson plans are written for an Advanced Placement high school chemistry teacher who would like to integrate the subject of clinical chemistry into the classroom. These plans are organized so the teacher can follow a lesson with consulting the literature review and other reference books for more information if necessary. The lesson plans are geared for the students to participate in "hands-on" laboratory experience. For example, in one lesson plan, the students use their own urine to test the analytes and their concentrations. Then, they will compare the results to a standard to ascertain how the analytes compare statistically with the standard and the other students' samples. Each lesson plan encounters a different aspect of clinical chemistry, so the students develop a broad knowledge base in the clinical field.

This thesis was written for several different reasons. First, the lesson plans can be an addition to the Advanced Placement (AP) Chemistry program after the Advanced Placement exam is completed. Since AP students are highly motivated and determined to accomplish the tasks involved in college-oriented general chemistry, exposure to clinical chemistry will allow students to expand their knowledge by integrating chemistry and biology.

Second, the students will learn many concrete level tasks and then further apply a formal cognitive level after mastering the concrete task. For example, students will analyze the glucose concentration in their urine. They can actually visualize the dipstick and verify if their glucose lies in the reference range. This task illustrates concrete reasoning skills. One step further on the formal reasoning level will be when the students apply a disease such as diabetes or reactive hypoglycemia to the level of glucose in the urine. In this learning process, the students can use this information and relate it to their own bodies.

The most important reason why this thesis was written is because the students can learn about the metabolism of their bodies. Many of the appliances we, as consumers, buy, have an instruction manual on how to make the appliance work at its optimum level. Our bodies have no instruction book attached to us when we are born on how to maintain good health or how to live life to its fullest. We are just supposed to know how to take care of our bodies and how metabolites work in our body. It is important for students at an age of growth and increasing responsibility to learn about how their bodies work. Therefore, the students can make a responsible decision based on their ideas and knowledge as well as the physician's knowledge of what is happening in their body.

These are the prominent reasons that clinical chemistry should be taught in the chemistry classroom. The students will enjoy these activities and take out more than they came in with at the beginning of this chemistry section. When the students can apply these medical tests to their own bodies, it is exciting because they are the center of discussion and each one of them is equally important when it comes to the human life.

EDUCATIONAL LITERATURE REVIEW

This section consists of a literature review about: (1) Piaget's theory of cognitive development stages, and (2) the relationships between formal reasoning, concrete reasoning, and chemistry.

Some students experience a transition in their mental development during their high school years. This transition involves the development of abstract reasoning, which is difficult for many students and even adults (Pallrand, 1979). This review investigates the cognitive abilities of high school chemistry students and the ways in which it is possible to facilitate the process of learning chemistry.

The Transition to Formal Thought as described by Piaget.

Chemistry is a very abstract subject whether it is encountered in high school or in college. For example, some abstract concepts taught in chemistry are: the mole, the element, a compound, the atom, and the electron. Currently, in order for students to learn abstract chemical concepts, they need to be thinking at the formal operational level. This mode of thinking is the final thinking level of Piaget's four steps in cognitive development and mental structure. Piaget postulated four stages of cognitive states, beginning with the sensori-motor intelligence, which lasts from birth to around one and a half to two years of age. The second stage is the pre-operational stage, which leads into the concrete operational stage at around eight years of age. The final cognitive state is the formal operational stage that develops between the ages of eleven and fifteen (Pallrand, 1979; Herron, 1975). A study conducted by Jackson found that approximately half of the fifteen year-olds did not demonstrate any formal thinking processes (Jackson, 1965). Furthermore, Renner and Lawson (1973) and Renner (1971) have found that as many as

half of the students enrolled in physical sciences classes at the college level are not operating at the formal thinking level (Pallrand, 1979).

The age ranges corresponding to Piaget's four stages of cognitive development coincide with the neurological maturation of the brain (Lawson, 1985). According to Epstein (1978), the brain actually grows in spurts that parallel the chronology of Piaget's cognitive development stages. Therefore, formal reasoning is impossible until some type of neurological maturation occurs, normally at either eleven or twelve years. However, Epstein's data has been questioned in terms of its validity (Lawson, 1985). It has been shown that stimulating the physical and social environment of students can lead to necessary neurological development (Lawson, 1985). When there is an absence of cognitive nourishment, students can usually overcome this lack with the appropriate schooling. Appropriate schooling encourages students to generate their own ideas, increase their curiosity and provide an atmosphere to think abstractly. The teacher is the person that can promote this development in the classroom since high school students are thinking at either the concrete operational stage or the formal operational stage (Pallrand, 1979).

The third stage of Piaget's developmental model is the concrete operational stage. Students of this stage relate to concrete objects and they are learned through sensory observation of examples and nonexamples. Examples of concrete objects are: a graduated cylinder, a thermometer, and a balance. Nonexamples of concrete objects are abstract concepts that cannot be learned through the five senses. Nonexamples are: the mole, the proton, and the molecule. By using these examples of concrete objects, the characteristics of the concrete concepts are made easier to understand. Then, the student uses these attributes to classify if other problems are examples or nonexamples of the concept (Cantu and Herron, 1978). Because concrete operational students relate to

concepts by direct experience, it is necessary to make the abstract concepts more concrete with the use of visual diagrams, illustrations, and models (Staver and Halsted, 1985). Since concrete concepts only require concrete reasoning to understand, it is only the content of the problem that the students learn and not how to extrapolate the problem one step farther (Beistal, 1975). Herron, (1975, p.148) composed a list of the competencies chemistry students at the concrete operational level are expected to do as listed in Table 1.

Table 1. Competencies of Chemistry Students at the Concrete Level that they Can and Cannot Do

Things students who have not reached formal level **CAN DO**	Things students who have not reached the formal level **CANNOT DO**
1. Follow a set of rules to find the empirical formula of a compound.	1. Understand why following the rules will result in the empirical formula.
2. Define an acid as any substance that will turn litmus paper red.	2. Define an acid as a proton donor or an electron acceptor.
3. Measure density by taking mass and volume.	3. Compare the densities of two or more substances.
4. Use factor-label to solve a problem in situations where the units provide an indication of the operations to be performed.	4. Use ratio and proportions to solve problems which will fit into a type that has been memorized.
5. Knowing the volume of the base needed to neutralize 1 g of acid, calculate the volume of base for any amount of acid.	5. Knowing the concentration of base and the volume needed to neutralize a given concentration of acid, calculate the volume of the acid.

The transition to formal thought requires the student to extend his/her knowledge beyond the present and the past and begin to think of the future possibilities (Herron, 1975). According to Beistal (1975), concrete students can become formal operational thinkers by careful experimentation and extensive careful questioning which leads the student to deduce principles. The formal student can apply mental operations to concepts, abstractions, and theories (Goodstein and Howe, 1978). He or she is no longer limited to

visual or personal experiences. Thus, the student can combine and understand a variety of ideas. Furthermore, the formal student can do proportional thinking and control variables, whereas the concrete student has a difficult time with these tasks. Also, he or she sees the necessity of "All other things being equal" (Herron,1975). Formal reasoning is a very important factor in cognitive development, although many adults are limited in their ability to use formal reasoning (Cantu and Herron, 1978; Tobin and Capie, 1981; Chandron, Treagust, and Tobin, 1985).

Although a student can be thinking at the formal operational level, he or she can revert back to the concrete operational level or pre-operational level when encountering a new idea or concept, according to Piaget (Herron, 1978). It is awkward for some students to change from concrete thinking to a formal thinking level, so they just go back to thinking in a concrete way, where it is comfortable. To illustrate, a swimmer who has just learned to swim is trying to breathe on the left side. The swimmer can do it, but it is very awkward and uncomfortable and it is easier to swim while breathing on the right side. Therefore, in a swim race, the swimmer will most likely breathe on the right side the whole time, even if it is more feasible to breathe on the left. In a similar way, when a student begins to use formal reasoning, it is awkward. During an exam, a student is likely to revert back to concrete reasoning because it has been practiced many times (Herron, 1978).

According to Herron (1978), people use the reasoning they have used successfully in the past. They need to describe and experience (concrete reasoning) before they explain the experience (formal reasoning). Many students need the experience as a foundation and then, they can provide meaning to the abstract, formal discussions. Therefore, it is necessary to introduce a concept with concrete reasoning and then follow through with the abstract logic for the formal operational students.

The Relationship Between Formal Reasoning, Concrete Reasoning, and Chemistry.

Chemistry is a subject that has many concepts which require advanced cognitive skills. Evidence from educational research suggests a variety of cognitive factors necessary for chemistry achievement. According to Chandran, Treagust, and Tobin (1987), there are four cognitive factors. These factors are: formal reasoning ability, prior knowledge, field dependence, and memory capacity. They found that formal reasoning and prior knowledge played significant roles in chemistry achievement for the three variables, which are laboratory applications, chemical calculations, and content knowledge (Chandran et al., 1987). Prior knowledge is the most important cognitive factor that has an effect on student's learning. Therefore, the authors proposed that if the students have the necessary prerequisites for the class, then they will achieve a higher understanding. Also, it is important that the instructor introduces the formal concepts in a concrete manner to insure that the learning occurs for a majority of the students.

While the short-term memory has no significant relationship to achievement in chemistry, the process of how information is stored in "chunks" may influence the success of the student (Chandran et al., (1987). The memory capacity is defined as how many schemes a student can store in the brain concurrently, according to Chandran et al., (1987). Students who have reached the formal level have a greater ability to "chunk" more information than students at the concrete level. Also, abstract thinkers are more intuitive on how they select information to be stored.

Goodstein and Howe (1978) conducted an experiment involving high school students at both the concrete and formal operational stages. One group of students used concrete props such as candies as atoms with toothpicks as bonds. This group used models to learn and understand the formal concept, stoichiometry. In addition, the teacher

used models as much as possible during the lectures. The other group had no concrete exemplars or models. The students were tested with the usual unit test to assess the qualitative understanding. Two questions were inserted at the end to discriminate between conceptual understanding versus mechanical manipulation of numerical data. These authors concluded that only the upper formal group of students benefitted from the models, while the lower formal and concrete students showed no significant difference. Also, it was found that when the upper formal students did not have the models, they learned at the same level as the concrete students. These results were not what the researchers predicted. They believe that concrete level thinkers cannot learn abstract concepts with any type of instructional teaching.

Another study by Herron (1978) on concrete models found contrary results to Goodstein and Howe. Herron's group taught a concept of an ideal gas using line drawings to illustrate a behavior of an ideal gas compared to its behavior with a real gas. Concrete operational students that studied this lesson averaged 61% on the test, while formal operational students averaged 72%. Then, it was taught to another group of students with no illustrations. In this case, the average for the concrete students was 51% and the average for the formal students was 66%. Thus, the illustrations improved the scores of both the concrete and formal operational students, but the effect seems to be slightly greater for the concrete operational students. In conclusion, it may be possible to use Piaget's theory to identify concepts which may be difficult for concrete operational students. If teachers are more aware of this learning development, then they may be able to restructure the course, so these abstract concepts are postponed until the student develops a foundation of the basic skills. Then, the concepts may be easier to learn for the concrete operational students.

Beistal (1975) also believes that the chemistry curriculum should be altered, so the concrete student could begin with concrete concepts such as phase equilibria for one and two component systems. Then, the student could progress to other properties such as solution theory (vapor pressure relationships and other colligative properties) before proceeding to the microscopic level. If the concrete student starts the semester with atomic structure, then the student is pushed far beyond his/her intellectual limit at the very beginning. This idea is a positive outlook towards learning chemistry in a different way which could be constructive for students who have not yet developed formal reasoning skills.

Since a substantial number of high school students do not operate at the formal level, many students are unlikely to learn abstract concepts meaningfully. Cantu and Herron (1978) conducted a study with "pseudoexamples" which use visual aids such as illustrations, diagrams, and models to illustrate abstract examples encountered in chemistry. Because formal thinking requires the student to use hypotheticodeductive reasoning about unseen entities, pseudoexamples are useful to provide direct information about a concept without the student having to discriminate between examples and nonexamples. Hypotheticodeductive reasoning is defined as a method to deduce the answer by a series of "trial and error" experiments. Thus, concrete students can learn abstract concepts in more of a concrete way.

In this study by Cantu and Herron (1978), there were three concrete and three abstract concepts used in this study. For the concrete concepts, simple devices were used to identify the important characteristics of the concepts which were not directly observable. On the other hand, for the abstract concepts, such as the ideal gases lesson, line drawings were used; in the isomers lesson, pictures of models were used; in the Bronsted acids and bases lesson, diagrams and chemical symbols were used.

Their results showed that the formal operational students were able to learn the concrete and formal concepts better than the concrete operational students. Cantu and Herron (1978) stated, "We do not believe that concrete operational students will learn these ideas as well as the formal operational students, and we do not believe that their level of understanding will be adequate unless the instructional procedures used to teach the concepts are carefully designed so that formal reasoning is not involved in the lesson." It seems that the question is whether the teaching instruction can narrow the gap between the concrete and formal students by being aware of the differences in concrete and formal thought processes. Also, pseudoexamples have been demonstrated to increase the achievement of the students at both intellectual levels for the comprehension of abstract concepts.

In summary, this literature review has shown that many of the concepts that are taught in chemistry require formal reasoning skills and a number of high school students have not acquired these advanced thinking skills. In order to be successful in chemistry, the teacher must assist students in their development of reasoning. Also, the teacher needs to practice teaching, so that the formal concepts can be understood by students who remain at the concrete operational level (Cantu & Herron, 1978). Researchers have demonstrated that the cognitive ability of the student is extremely important when investigating how well a student can understand concepts in chemistry.

CHEMICAL LITERATURE REVIEW

INTRODUCTION TO THE CLINICAL LABORATORY

The sections in the chemical literature review are designed to complement the lesson plans. The same names correlate to both the chemical literature review and the lesson plans. The lesson plans are as follows: (1) Introduction to the Clinical Laboratory, (2) Diagnosing Glucose Metabolites in the Blood and Urine, (3) The Blood Gases, (4) Analysis of Analytes and Cells in the Urine, and (5) Antigen and Antibody Reactions.

Clinical Chemistry is defined as the chemistry of human health and disease in connection with the management of patients (Steadman's Medical Dictionary, 1990). The function of the clinical chemistry laboratory is to perform qualitative and quantitative analyses on body fluids such as blood, urine, and spinal fluid. The analytical values obtained can then be compared with the appropriate reference range values to determine if there is something wrong with a patient.

The focus of this section of the review consists of: (1) quality control and quality assurance, (2) a review on the SI units involved in clinical chemistry and (3) a review of the digestion of carbohydrates, proteins, and fats.

Quality Control and Quality Assurance

The clinical laboratory plays an important role in the analyses of various body fluids. For the results to be useful to a physician for diagnosing and treating disease, the analytical tests must be performed as accurately as possible (Tietz, 1987). Good instrumentation and sound analytical methods are necessary to achieve the most accurate results. There are basic principles and procedures to follow while examining body fluids in the clinical laboratory. Some essential factors are the purity of reagent solutes and

solvents, quality of the containers, reliability and quality of measuring devices and methods, appropriate choice of separative methods and devices, and observance of the safety procedures (Tietz, 1987).

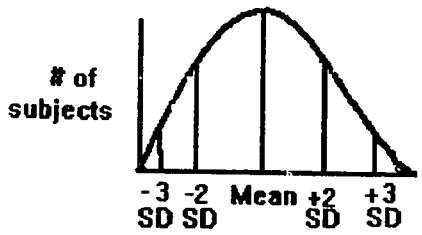
Quality assurance is defined as "a broad spectrum of plans, policies, and procedures that together provide an administrative structure for a laboratory's effort to achieve quality goals", (Tietz, 1987). The term "Quality Control" (QC), is often used to represent those techniques and procedures that monitor performance characteristics. These are usually quantitative techniques that monitor particular sources of errors, estimate the magnitude of errors, and indicate to the laboratory personnel if or when quality has diminished (Tietz, 1987).

The ideal analytical method is accurate, precise, sensitive, and specific. It gives a correct result (accurate) that is the same value even after repeating the procedure (precise). Also, this method must measure low concentration of analytes (sensitive) and not be subject to interference by other substances (specific) (Marshall, 1992). When conducting a test, a standard should always be tested along with an unknown. By utilizing a standard, the clinician can recognize more readily an abnormal sample. It is also important to use a reference standard to monitor precision and accuracy of the method.

Usually, the clinician is directed to perform a group of related tests on a patient that will help determine the diagnosis. These "biochemical profiles" can aid in narrowing the possibilities of certain diseases or conditions. When a biochemical test is obtained, the following points must be taken into consideration: "Is the test result normal?", "Is the value significantly different from the reference range?", "Is the test result consistent with the clinical findings?". After these questions are answered, then the value obtained should be compared to the normal values. The normal range is usually a distribution of values

from repeated measurements of the same quantity and is described by the bell-shaped Gaussian curve, shown in Figure 1 below.

Figure 1. Gaussian Curve



Normal statistical theory predicts that approximately 95% of the values will lie within the range of + two standard deviations from the mean; the remaining 5%, equally split, will lie outside this range (Marshall, 1992). The normal range of an analyte has severe limitations because it only identifies the range of values that can be expected to occur most often in the individuals who are comparable to those in the population for whom the range was derived (Marshall, 1992). Instead of one normal value, a reference range of values is used. The greater the difference from its reference range, the more likely a test result is associated with a pathological process. If the result is consistent with the value of a specific disease or symptom, it is evidence in favor of the clinical diagnosis. For example, if a patient has a blood glucose level greater than the reference range (70-120 mg/dL), then the patient most likely has hyperglycemia.

The biochemical tests in the clinical laboratory are important because they can be used for several different management stages of diseases that have an obvious metabolic bases. These four stages are called diagnosis, prognosis, monitoring, and screening (Beeler and Catrou, 1988). Diagnosis is the confirmation or rejection of the clinical disease. It is based on the patient's history combined with an examination. Prognosis is the information regarding the likely outcome of the disease. The biochemical tests are used primarily for diagnosis and may also provide prognostic information. Biochemical

tests, such as the serum glucose assay, can also predict the risk of a particular condition such as diabetes. Monitoring is looking at the natural history or the response to the treatment. It follows a course of an illness and also monitors the effects and complications of treatment. Screening is the detection of subclinical (early stage) of the disease. It is used to see whether a disease is present subclinically.

A sample must be collected and transported to the laboratory according to a specified procedure if the data are to be of clinical value. The record of the patient's history is very important with information including name, sex, age, date of birth, ward, requested doctor, clinical diagnosis and problem, tests requested, type of specimen, date and time of the sample (Beeler and Catrou, 1988). For the transportation of HIV or hepatitis samples, great care must be provided. When a sample needs water, the clinical laboratory must make sure that the water is "pure" and decontaminated, otherwise the test will not result in complete accuracy.

Units and Measurement in the Clinical Laboratory

All quantitative measurements must be expressed clearly in defined units that are accepted by the scientific community. A meaningful measurement requires a number followed by appropriate unit(s). The units identify the dimension - mass, volume, or concentration of a measured property. The number indicates how many units are contained in the property. The modern system in the clinical laboratory uses system international units (SI units), which is the measurement of choice by most countries (Tietz, 1987). One of the advantages of the metric system is that it is a decimal system and it is based on powers of ten. For example, there are 10^3 grams in a kilogram, so the prefix "kilo" means one thousand. The SI system uses grams, meters, and liters, whereas the U.S. system uses pounds, yards, and gallons (Kaplan et al., 1988). Some units outside the

SI system continue to be important and useful in particular applications. For example, the liter is the reference volume in the clinical laboratory instead of the SI unit of cubic decimeter. One particular unit of importance in the clinical laboratory is the measurements for an amount of substance expressed as mole (mol). The conventional units are more familiar for the amounts of constituents in body fluids, which have generally been reported as substance concentrations rather than mass concentrations (Kaplan et al., 1988). For example, 2.5 mmol/L instead of 10.0 mg/dL for calcium and 3.9 mmol/L instead of 70.0 mg/dL for glucose are used (Tietz, 1987).

Review of the Digestion of Carbohydrates, Proteins, and Fats

For the students to understand the abnormal symptoms in the metabolic diseases, it is necessary to review how food is utilized in the body and how the body excretes its waste products such as carbon dioxide (CO_2), bicarbonate (HCO_3^-), and nitrogen products such as urea (CH_4N_2O) and uric acid ($C_5H_4NO_3$). Also, oxygen (O_2) is necessary for the metabolism of food throughout the body. The digestion of carbohydrates, proteins, and fats are described below.

The digestion of carbohydrates starts in the mouth where the enzyme called salivary amylase breaks down the starch into maltose, glucose, and smaller units of starch polymer called starch dextrins (Montgomery et al., 1990). Salivary amylase also protects the teeth and prevents bacteria build up on the teeth. Then, after swallowing, the food bolus meets a high acidic concentration when it enters the stomach and is further broken down by pancreatic amylase secreted by the pancreas to maltose, D-glucose, sucrose, isomaltose, dietary lactose and α–limit dextrins. Then, the brush border cells hydrolyze the disaccharides into fructose, glucose, and galactose by enzymes called α D-glucosidases in the cell membrane. These monosaccharides are formed in the lumen and

pass into the portal blood system where it passes into the capillaries of the small intestines. Then, they pass into the hepatic portal vein and further into the capillaries of the liver. They are transported into the liver and then to the remainder of the body. Glucose proceeds into either aerobic or anaerobic glycolysis, which is known as aerobic and anaerobic cellular respiration. Aerobic glycolysis produces 24 adenosine triphosphate (ATP) molecules, 6 carbon dioxide molecules and 6 water molecules, whereas anaerobic glycolysis produces 2 ATP molecules and 2 lactate molecules (Montgomery et al., 1990). Then, the metabolic wastes, which are CO_2 and water (H_2O), are excreted in the air exhaled by the lungs. The kidneys are responsible for removing H_2O, HCO_3^-, sodium ions (Na^+), ammonium ions (NH_4^+) and urea (CH_4ON_2) from the blood that passes through the kidneys (Kraus, 1984).

The digestion of dietary proteins starts in the gastrointestinal tract where they are broken down into amino acids by proteolytic enzymes. The first proteolytic enzyme to act in the stomach is called pepsin which is activated by its zymogen, pepsinogen and hydrochloric acid. Pepsinogen is activated by the release of the hormone, gastrin. Gastrin also acts on the pareital cells to release hydrochloric acid. These enzymes (pepsin and trypsin) digest the proteins into mostly large polypeptides. In the small intestines, secretin, a hormone in the pancreas, releases the protein-free electrolyte solution that has a high concentration of HCO_3^-. This solution neutralizes the acidic concentration from the stomach and increases the pH of the small intestines to approximately 7.5 - 8.5. Then, the enzymes trypsin, chymotrypsin, and elastase cleave polypeptides at the internal site of the polypeptides, whereas carboxypeptidases cleave the amino acids from the carboxyl ends of the polypeptide chains. Then, the peptides are hydrolyzed by peptidases located in the absorptive cells of the small intestine so that only amino acids are released into the portal blood system (Montgomery et al., 1990). For example, glutamine, the most abundant and

most important amino acid, is transported to the liver and may be hydrolyzed by liver glutaminase to yield NH_4^+. These NH_4^+ ions can be used by the liver for urea synthesis. Urea is filtered through the kidney and excreted into the urine. The carbon skeletons of the amino acids may be used to produce energy.

The breakdown of fatty acids begins in the small intestine. The liver releases bile into the small intestines to break down the fatty acids into smaller particles. The small fatty acids are absorbed into the blood stream and transported to the liver by the portal system since they are water soluble. Then, they are used for building material. The larger fatty acids are absorbed into the lymphatic system. A protein carrier is attached to the fatty acid as it circulates through the lymphatic system. This process dilutes the fatty acids while they circulate throughout the body. The small fatty acids undergo a process called β-oxidation and the end product is acetyl CoA, which can continue through the Kreb's cycle to produce energy (in the form of ATP), H_2O and CO_2. H_2O and the CO_2 are exhaled through the lungs for excretion, while the kidneys also excrete H_2O (Montgomery et al., 1990).

CLINICAL TESTS AND DISEASES

This section of the clinical chemistry review consists of: (1) clinical tests conducted to ascertain the normal physiology and diseases associated with them, and (2) the symptoms and the causes of the disease. Also, there is a glossary of medical terms for the reader.

There are many tests to determine whether or not a patient has a specific disease. It is important to be thorough in the analysis of the test to reinforce its validity. Clinical laboratories are extremely important to ensure the accurate diagnosis. Therefore, interpretation of what the test results mean are required. The laboratory needs several tests to ascertain the roles of metabolites in the body at a specific time. Although some tests are not completely accurate for diagnosing a disease by themselves, these tests along with other diagnostic tests can be valid for determining a disease. In addition to the clinical laboratory tests, it is important to consider the symptoms of the patient when diagnosing the disease.

DIAGNOSING GLUCOSE METABOLITES IN THE BLOOD AND URINE

In this section, the glucose levels in the blood and the urine will be discussed. The normal values for glucose are for an adult, 70-120 mg/dL and for a child, 40-80 mg/dL (Blick and Liles, 1988). There should be no glucose present in the urine because it should be filtered through the kidneys and absorbed back into the blood stream. Otherwise, if glucose is present in the urine, then this observation indicates there is an excess amount of glucose in the bloodstream. When the amount of glucose is greater than 120 mg/dL, then the person is diagnosed as hyperglycemic. Hyperglycemia can be a symptom of many diseases such as diabetes and renal failure. If there is a low amount of glucose in the blood stream, then the person is diagnosed with hypoglycemia. There are two major types

of hypoglycemia, reactive hypoglycemia and fasting hypoglycemia, that are known to lower blood glucose levels. For hyperglycemia and hypoglycemia, one should conduct tests involving glucose metabolism and any factor that affects the rate of metabolism such as the levels of insulin or glucagon in the plasma.

Tests for Glucose in the Urine

There are two tests to ascertain whether glucose is in the urine. The most common test for glucose in the urine is the dipstick method. In addition, there are reagent tablets by Acetest (Ames) that may be used instead of the dipsticks. Diabetics using the dipstick tests must recognize that they are testing their whole blood sugar rather than plasma or serum glucose, which is about 10-15% lower because of the difference in water content (Kaplan et al., 1988; Marshall, 1992). Therefore, they should carefully analyze the test strip. Currently, diabetics can use a small reference meter to determine the concentration of glucose in the blood, which is more accurate than the dipstick method (Kaplan et al., 1988).

The two common dipstick brands are Ames and Boehinger Mannheim (Beeler and Catrou, 1988). The dipstick is impregnated with glucose oxidase, peroxidase, buffers and a chromogen (Beeler and Catrou, 1988; Kaplan, Szabo, and Opheim, 1988). Glucose oxidase is an enzyme that in the presence of oxygen converts glucose to gluconic acid. Then, peroxidase, also an enzyme, converts hydrogen peroxide to water, while the chromogen is being oxidized, displayed by a change in color. The chromogen is colorless when glucose is in the reduced state and colored when glucose is oxidized.

The reaction scheme is as follows (Kaplan et al., 1988; Blick and Liles, 1985):

(1) β–D-glucose + O_2 ----------> gluconic acid + H_2O_2

(2) H_2O_2 + reduced chromogen ----------> 2 H_2O + color in chromogen

There are two compounds that can act as reduced chromogens.

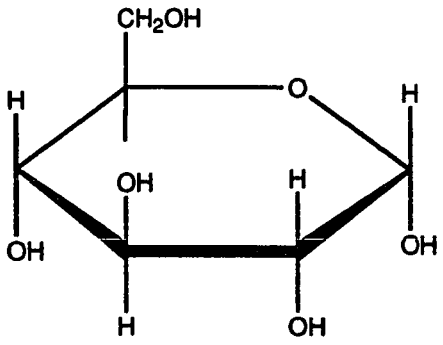
(3) KI (reduced, colorless) ----------> I_2 (oxidized, brown)

(4) o-tolidine (reduced, colorless) --------> dehydrogenated o-tolidine (oxidized, blue)
as a hydrogen donor

The glucose concentration is proportional to H_2O_2 produced in reaction (2).

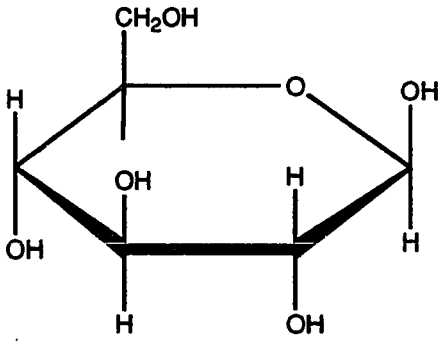
However, the urine glucose determination by dipstick is an insensitive procedure for satisfactory screening by itself (Beeler and Catrou, 1988). A blood glucose determination is also required for sufficient screening. The reason for the error is due to the different glucose conformations. When the glucose is in the urine, it can be in α-glucose (36%) and β-glucose (64%), which structures are shown in Diagram 1 and 2 below.

Diagram 1. α-glucose



α - glucose

Diagram 2. β-glucose



β - glucose

The sample needs at least two hours to equilibrate a mixture that is predominent in β-glucose before an accurate reading can occur (Kaplan et al., 1988). Unfortunately,

various serum components such as uric acid, ascorbic acid, and glutathione compete with the chromogen as reducing agents, which cause falsely low values by interference (Kaplan et al., 1988).

Test for Ketone Bodies in the Urine

Another dipstick method is used to check the presence of ketone bodies in the urine. This test is important when screening for uncontrolled diabetes, which is when the patient either does not know of the onset of the disease or does not take proper care maintaining an appropriate blood sugar level. Diagrams 3, 4, and 5 show the three ketone bodies, which are acetoacetate, acetone, and β- hydroxybutyrate. These ketone bodies are products from the oxidation of fatty acids (Beeler and Catrou, 1988).

Diagram 3. Acetoacetate

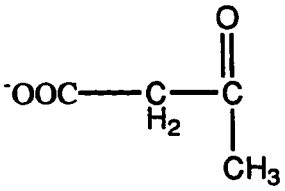


Diagram 4. Acetone

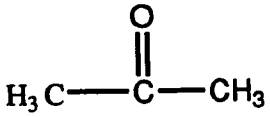
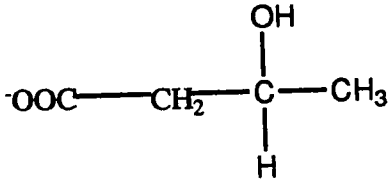


Diagram 5. β-hydroxybutyrate



The dipstick is impregnated with sodium nitroprusside (nitroferrocyanide) and an alkaline buffer. Sodium nitroprusside is important because it is the compound on the dipstick that acetoacetate and acetone react with to verify their presence in the urine. In the presence of acetoacetate and acetone, a lavendar color is produced that is compared with a color chart. β-hydroxybutyrate is the only ketone body that does not react with sodium nitroprusside, but it is still present in the urine. A small concentration of

acetoacetate is 10 mg/dL, moderate concentration is 30 mg/dL and a large concentration is 80 mg/dL (Kaplan et al., 1988).

Test for Plasma Glucose Levels

Oral Glucose Tolerance Test (OGTT)

A test given that measures a patient's plasma glucose levels is called the Oral Glucose Tolerance Test (OGTT). Before the test, the patient must fast at least ten hours and no more than sixteen hours. The adult patient should be placed on a carbohydrate diet with at least 150 g/day before the fasting. For a child, it is based on the body weight (1.75 g/kg ideal body weight) (Blicks and Liles, 1988).

The first part of the OGTT, as a fasting blood sugar level for an adult, is to ingest 75 g of glucose dissolved in water (25 mg/dL). The glucose is consumed in a period of approximately five minutes. Blood samples are collected at thirty minute intervals for two hours (Blicks and Liles, 1985). It is preferred that the blood collected in the test always comes from the same source. For example, if one collects the blood from the vein, then the next collection of blood should also be from the vein. If possible, venous blood should be collected. The peak concentration is normally reached between 30 - 60 minutes. After two hours, blood levels should drop slightly and then return to normal in approximately three hours. If the patient is hypoglycemic, then the test is extended to four to five hours.

Two Hour Postprandial Test

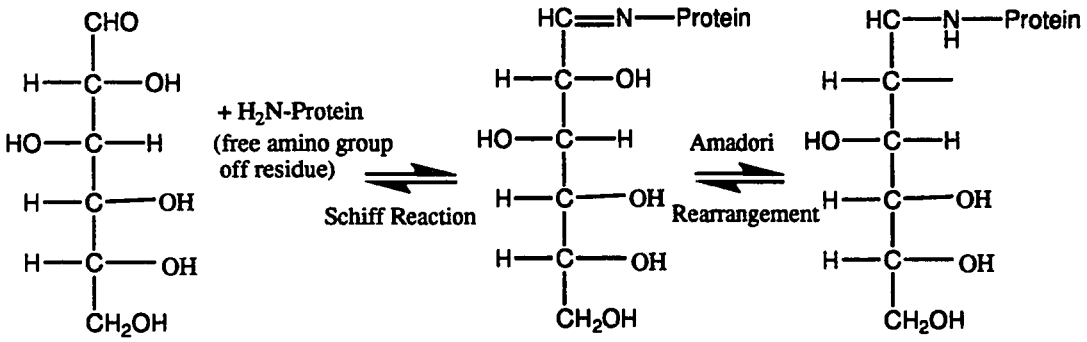
Another test to screen glucose levels for diabetes is called the Two Hour Postprandial Test. The patient is given a glucose load or eats a meal with 75-100 g carbohydrates. A two hour postprandial blood specimen is drawn and the glucose of the

blood sample is determined. In normal patients, the two hour postprandial values should approximate the fasting glucose level, which is below 70 mg/dL (Blick and Liles, 1988). In older individuals, the delay in insulin release may yield values slightly higher than the fasting glucose level (Kaplan et al., 1988; Blick and Liles, 1985).

Test for Glycosylated Hemoglobin

Glycosylated hemoglobin can show diabetic control over a longer period of time such as two to three months (Marshall, 1992). In the body, D-glucose reacts nonenzymatically with the free amino residues in proteins. In hemoglobin, glucose reacts with the free amino groups of the lysine residues or with the amino terminal valine on the hemoglobin β chains to form a Schiff base (an aldimine), which may undergo an Amadori Rearrangement to a more stable ketoamine. Reaction 1 below describes this reaction mechanism (Montgomery, Conway, and Spector, 1990).

Reaction 1. Glycosylated Hemoglobin Reaction



D-glucose aldimine ketoamine

This reaction occurs continuously during the life span of the red blood cell and can be detected by analyzing hemoglobin. The degree of hyperglycemia over a given time period is reflected in the amount of glycosylated hemoglobin in the blood sample and

therefore, presents an important diagnostic tool. It shows how well the blood glucose level is controlled over a period of six to eight weeks.

The glycosylated hemoglobin is determined by lysing a red blood cell sample that have been maintained in isotonic saline for a specified time to remove any free glucose in the cell (Montgomery et al., 1990). The lysed cells are centrifuged to remove cellular debris and the clear supernatent is chromatographed on an ion exchange column. The eluate is measured spectrophotometrically at 410 nm. Hemoglobin and its derivatives resolve as two peaks, HBA_{1a+b} and HBA_{1c}. $HBA1_c$ is formed from the condensation of glucose with its amino terminal valine in the β-chains of hemoglobin A (Montgomery et al., 1990). The integration of the peaks determines the components of these peaks. HBA_{1c} is usually 5-9% of the total hemoglobin in normal individuals. In persons with uncontrolled diabetes, this level is usually elevated up to a value of 20% of the total hemoglobin (Marshall, 1990). This test is helpful when there is a discrepancy between a patient history, blood glucose and urine glucose because this method effectively measures the concentration of glucose over the previous two to three months (Marshall, 1990).

Interpreting Glucose Test Results

The previous tests measure glucose levels in the blood and urine. Each test has a normal range to two standard deviations. When the concentration is higher or lower than the normal range, then the patient can be diagnosed for a disease and treated. Table 2 lists the plasma or serum glucose reference ranges (Brick and Liles, 1988).

Table 2. Plasma Glucose Levels in Adults and Children

serum or plasma glucose (mg/dL)	Adult	Child
normal	70 - 120	40 - 80
low	< 40	< 30
high	>700	> 300
whole blood "true glucose"	60 - 100	20 - 6

Hyperglycemia

Hyperglycemia is a disorder in the carbohydrate metabolism, where there is a raised plasma glucose concentration. A disease that is associated with hyperglycemia and affects 5% of the American population is called diabetes mellitus (Blick and Liles, 1988). It is the seventh leading cause of death in America (Kaplan et al., 1988). Diabetes mellitus may not be recognized immediately and the disease could eventually display serious secondary effects such as cataracts, kidney disease, and circulatory problems if it is not treated. According to Blicks and Liles (1988), diabetes mellitus may be defined as a genetically and clinically heterogenous group of disorders associated with an complete lack or relative lack of insulin.

There are three types of diabetes mellitus: (1) insulin-dependent diabetes mellitus (IDDM), (2) noninsulin-dependent diabetes mellitus (NIDDM) and (3) other types such as gestational diabetes, and pancreatic diabetes (Blick and Liles, 1985).

Insulin-Dependent Diabetes Mellitus

Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disorder in which antibodies destroy the cells where insulin is produced. Insulin is a small polypeptide that affects glucose metabolism by promoting the entry of glucose into the body cells (other than hepatic) (Beeler and Catrou, 1988). It is produced by the beta cells in the pancreatic islets of Langerhans (Marshall, 1992). The etiology suggests genetic, environmental or acquired factors, and association with certain histocompatibility antigens (HLA) on chromosome 6 (Brick and Liles, 1985). The onset of IDDM is sudden and occurs most often in childhood due to determinants such as abnormal immune response, viral infection, decrease in insulin, and islet cell antibodies (Marshall, 1992). It is commonly known as junvenile-onset diabetes mellitus.

The symptoms of IDDM include polyuria, polydipsia, rapid weight loss, ketonuria, hyperglycemia, metabolic acidosis, hypertryglyceridemia, and glycosuria (Marshall, 1992). The final stages of the disease result in stroke, myocardial infarction, loss of eyesight, renal failure, and neurological defects (Kaplan et al., 1988).

Non-Insulin Dependent Diabetes Mellitus

Non-insulin dependent diabetes mellitus (NIDDM) is also called adult-onset diabetes because most of the cases occur after the age of forty years, although it may occur at an earlier age (Blicks and Liles, 1988). This group of patients are not insulin dependent or ketosis prone, although there are those who may require insulin for the control of hyperglycemia. The serum insulin levels may be depressed, normal or elevated.

Patients with NIDDM show resistance to insulin, which is thought to be due to a reduction in the number of insulin receptors on the cell membrane or to decreased affinity of the insulin receptor (Marshall, 1992). About 60-90% of NIDDM subjects are obese

and it is usually a strong familial inheritance. The symptoms for NIDDM are similar, but not as acute as the symptoms for IDDM. NIDDM can be treated with change in diet, weight reduction and increased physical activity, which will increase the insulin levels, increase the number of insulin receptors, and improve the insulin effectiveness (Blick and Liles, 1985).

To ascertain whether a person has diabetes mellitus, the glucose metabolism tests described previously show the following results:

(1) Oral Glucose Tolerance Test	> 200 mg/dl
(2) Fasting Plasma Glucose (on more than one occasion)	> 140 mg/dl
(3) 2 Hour Postprandial	> 200 mg/dl
(4) Glucose in Urine (dipstck)	positive
(5) Ketone Bodies in Urine (dipstick)	positive
(6) Glycosylated Hb	> 7%

These are the tests performed to diagnose a patient with a possible case of diabetes mellitus.

Hypoglycemia

Hypoglycemia is defined as the rate of glucose absorbed into the blood is less than the rate of its uptake into the tissues (Marshall, 1992). Lack of dietary carbohydrates alone does not cause hypoglycemia; it is usually due to a decreased hepatic glucose release rather than to an increased tissue uptake. Hypoglycemia occurs when blood glucose levels fall below 60 mg/dL, according to Kaplan et al. (1988). Adults may lose

consciousness when the blood glucose levels drop below 40 mg/dL. Irreversible brain damage can occur if hypoglycemia persists for more than four minutes (Kaplan et al., 1988). Merinee and Tyson defined hypoglycemia in adult men that fasted for 24 hours to have a level below 55 mg/dL and in women, a level below 35 mg/dL (Beeler and Catrou, 1988).

Two major types of hypoglycemia are called reactive hypoglycemia and fasting hypoglycemia. Reactive hypoglycemia occurs when the low blood glucose concentrations result because of a stimulus, such as a meal. Fasting hypoglycemia occurs within several hours after ingesting a meal.

Reactive hypoglycemia

One type of reactive hypoglycemia is drug-induced hypoglycemia. Persons with IDDM might either inject too much insulin or miss a meal, which would cause hypoglycemia. Also, the sulphonylureas, which are drugs used to treat hyperglycemia in NIDDM subjects, can cause hypoglycemia. Furthermore, reactive hypoglycemia can result in patients treated with β-adrenergenic blocking drugs in conjunction with starvation or exercise (Marshall, 1992). Insulin and drug-induced hypoglycemia are heightened by alcohol. Alcohol increases the insulin release in response to a glucose load, which may lead to post-prandial reactive hypoglycemia (Marshall, 1992).

Reactive hypoglycemia is exaggerated in the early stages of diabetes mellitus or after overindulgence of alcohol. It is seen in postgastrectomy patients or in mild diabetes mellitus (Beeler and Catrou, 1988). There is not yet a uniformly demonstrable cause for all hypoglycemias and it is not progressive. It requires only dietary treatment with psychological support. The five hour glucose tolerance test differentiates between reactive and fasting hypoglycemia (Beeler and Catrou, 1988; Marshall, 1992).

Fasting hypoglycemia

Fasting hypoglycemia occurs beyond five hours after the last meal containing carbohydrates. It is more serious than reactive hypoglycemia (Beeler and Catrou, 1988). There are a variety of causes to this disease such as inappropriate insulin self-administration, deficiency of glucagon (raises blood glucose), excessive production and release of insulin by tumors (insulinomas), inadequate glycogen stores, and inadequate glycogenolysis (breakdown of glycogen) (Beeler and Catrou, 1988; Kaplan et al., 1988). Although uncommon, insulinomas are an important cause of severe fasting hypoglycemia. They are tumors of the insulin-secreting β-cells of the pancreatic islets. Other diseases that result in fasting hypoglycemia are hepatic and renal disease (rare), endocrine disease, glycogen storage disorders, and various forms of neonatal hypoglycemia (Marshall, 1990).

During a seventy-two hour fast, almost all insulinoma patients develop clinical hypoglycemia and severe symptoms such as unconsciousness and hypoglycemic shock. Blood samples should be collected every four to six hours during any clinical hypoglycemia episode. Since insulin induces hypoglycemia, a plasma C-peptide concentration can be measured. C-peptide is released from the pancreas in equimolar quantities with insulin, so it can be a valid measurement of the amount of insulin released from the pancreas. C-peptide is cleaved when the insulin is purified (Marshall, 1990). In normal individuals, hypoglycemia suppresses endogenous insulin and C-peptide secretion. Patients with insulinomas have a C-peptide concentration of greater than 1.2 μg/L, which implies continuing, autonomous insulin secretion (Marshall, 1990).

The clinical features for severe hypoglycemia are as follows: anxiety, detachment, dizziness, blurred vision, confusion, amnesia, weakness, coma, sweating, palpitations, diplopia, convulsions, and huge hunger (Marshall, 1990; Blick and Lile, 1985; Kaplan et

al., 1988). To determine if a person has hypoglycemia, the following tests are completed, which are listed in Table 3.

Table 3. Plasma Glucose Levels for Several Glucose Tests

Serum or plasma glucose levels		
(1) Fasting plasma glucose level	Men- < 55 mg/dL	Women-< 35 mg/dL
(2) 2 hour postprandial	Reactive - < 60 mg/dL	Fasting - normal
(3) 5 hour postprandial	Reactive - <60 mg/dL	Fasting - < 60 mg/dL
(4) C-Peptide test	Reactive - normal	Fasting - > 1.2 μg/L

THE BLOOD GASES

In this section of the chemistry literature review, the blood gases, which include the partial pressure of carbon dioxide (pCO_2) and the partial pressure of oxygen (pO_2), along with the hydrogen (H^+) ion concentration will be discussed in addition to the carbonic acid-bicarbonate buffer in the body. The partial pressure is the pressure that each gas in the blood contributes to the total pressure. The blood gases, pCO_2 and pO_2, are the partial pressures for CO_2 and O_2 dissolved in the blood. When there is an acid-base disturbance in the body such as vomiting, hyperventilation, or diabetes, then a respiratory or metabolic disorder will occur (Marshall, 1992). There are four types of acid-base disturbances which affect the concentration of pCO_2 and pO_2. They are respiratory acidosis, respiratory alkalosis, metabolic acidosis, and metabolic alkalosis (Marshall, 1992; Blick and Liles, 1988; Kaplan et al., 1988).

First, the metabolism and normal physiology of the blood gases will be reviewed. This discussion will also include how the lungs and the kidneys play significant roles in maintaining the balance of CO_2 and H^+ ion in the blood.

During energy-yielding oxidative metabolism, acid is generated from CO_2. The normal amount of CO_2 in the body is equivalent to at least 15 mol H^+ per day. Although CO_2 is not an acid, it can undergo hydration to form a weak acid, carbonic acid (H_2CO_3) in the presence of H_2O. Reaction 2 below describes this reaction (Marshall, 1992).

Reaction 2. Carbonic Acid / Bicarbonate Buffer Equation

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+(aq) + HCO_3^-(aq)$$

CO_2 is removed by expired air. In healthy people, the mechanisms controlling pulmonary ventilation respond in such a way that the rate of CO_2 excretion is equivalent to the rate of H^+ production. The normal values of the blood gases are: 4×10^{-8} M dissolved CO_2, 1×10^{-3} M H_2CO_3, and 25×10^{-3} M HCO_3^-. HCO_3^- is the major transport of CO_2 in the body (Larson, 1991).

The homeostatic mechanisms for H^+ and CO_2 are very efficient. The normal pH of the body is about 7.40, however temporary imbalances from the pH range 7.38-7.44 can be absorbed by buffering (Beeler, 1988). A buffer is a mixture of a weak acid and the salt of a base (a conjugate base pair). It has the capacity to resist change of $[H^+]$ when a strong acid or base has been added to it. Body buffers are the first defense of the body against changes in the $[H^+]$. The buffers in the body consist of carbonic acid-bicarbonate pair (H_2CO_3 / HCO_3^-) (one-half of the whole buffering capacity), hemoglobin (Hb) buffer (which contributes about one-third), plasma proteins, and monohydrogen phosphate-dihydrogen phosphate conjugate pair ($HPO_4^{2-}/H_2PO_4^-$) (Beeler, 1988).

The buffer that will be the topic of discussion in this review is the H_2CO_3 / HCO_3^- pair. It is in equilibrium with other buffer systems in the body. From Reaction 2 above, after H_2CO_3 is generated, it can dissociate into H^+ and HCO_3^-. The pH is proportional to the ratio of $[HCO_3^-]$ to $[H_2CO_3]$, according to Reaction 3 below.

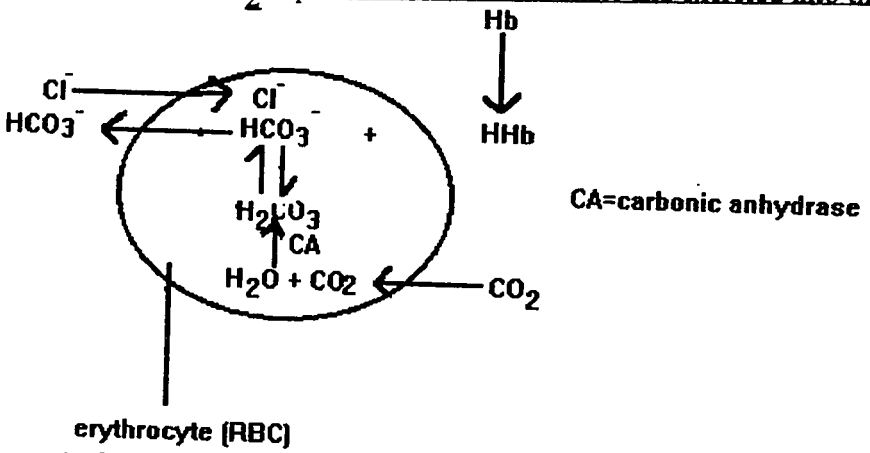
Reaction 3. Henderson-Hasselbach Equation for the Carbonic Acid / Bicarbonate Buffer

$$pH = pK + \log [HCO_3^-] / [H_2CO_3]$$

In the arterial blood, the ratio of $[HCO_3]$ / $[H_2CO_3]$ is 20:1. If this ratio should increase or decrease, then either a respiratory or metabolic disturbance will occur.

To maintain this ratio, the lungs and the kidneys are responsible in eliminating H_2CO_3 and in generating HCO_3^-, respectively. In the capillary beds, CO_2 diffuses into red blood cells and combines with H_2O to form H_2CO_3; this reaction is catalyzed by carbonic anhydrase. Then, H_2CO_3 dissociates to form H^+, which are buffered by Hb and HCO_3^-. Then, they diffuse out of the red blood cell. To maintain electrochemical neutrality, chloride ion (Cl^-) diffuses into the cell. In the alveoli, the process is reversed; CO_2 is produced from HCO_3^- and is excreted into the air (Marshall, 1992). This process is shown in Figure 2 summarizes this reaction scheme (Marshall, 1992).

Figure 2. Process of how CO_2 is produced from bicarbonate and excreted into the air.



Instead of eliminating H_2CO_3 in the lungs, the HCO_3^- is generated in the kidneys. Excess H^+ can only be eliminated from the body by the renal route, so the kidneys are of major importance when there is an acid build-up in the body. This process occurs on the luminar surface of the renal tubular cells, which is impermeable to HCO_3^- and therefore, direct absorption of HCO_3^- cannot occur. Essentially all of the filtered HCO_3^- is reabsorbed into the plasma. Inside the renal tubular cells, H_2CO_3 is formed by CO_2 and H_2O. This reaction is catalyzed in the kidneys by the enzyme, carbonic anhydrase. The H_2CO_3, then dissociates to give H^+ and HCO_3^-. The HCO_3^- ions pass across the basal

border of the cells into the interstitial fluid. The H^+ ions are secreted across the luminal membrane in exchange for Na^+, which accompany HCO_3^- into the interstitial fluid (Marshall, 1992; Zilva and Pannall, 1984). The formation of HCO_3^- and H^- is promoted by their continuous removal and by the presence of carbonic anhydrase.

In the tubular fluid of the kidneys, H^+ combine with HCO_3^- to form H_2CO_3, most of which dissociates into CO_2 and H_2O (Zilva and Pannall, 1984). Then, some of the carbon dioxide diffuses back into the renal tubular cells, while the remainder is excreted into the urine. In the kidneys, this process results in the reabsorption of the filtered HCO_3^-. With the aid of healthy lungs and kidneys, the homeostatic mechanisms for $H^+$and CO_2 can control the temporary imbalances in the plasma pH (Blick and Liles,1988).

If the CO_2 levels are either raised or lowered, it affects the pH. These changes result in respiratory acidosis or respiratory alkalosis, respectively. When the HCO_3^- concentration is raised, the disorder is called metabolic alkalosis. On the other hand, if the HCO_3^- concentration is lowered, then metabolic acidosis occurs.

Respiratory Acidosis

Respiratory acidosis occurs when there is insufficient O_2 present in the lungs. Thus, the pCO_2 builds up in the blood because the lungs cannot excrete all the CO_2 that is present in the blood. Therefore, more H_2CO_3 is formed, which lowers the pH. The $[HCO_3^-]$ is slightly increased or normal. Then, all of the blood gases accumulate in the plasma.

The causes of respiratory acidosis are diseases that block the passage of O_2 to the lungs such as: pulmonary edema, emphysema, broncho constriction (asthma), pneumonia, and congestive heart failure (Blick and Liles, 1988; Beecher and Catrou, 1992; Marshall,

1992; Jackson, 1991). Other causes of this disorder include a drug overdose on morphine (a respiratory depressant) or sedatives. Depression of the respiratory center by a cerebral trauma or a tumor can cause respiratory acidosis. Neuromuscular diseases such as poliomyelitis, botulism, and tetanus as well as neurotoxins can also be sufficient causes of this disorder (Marshall, 1992).

For treatment of respiratory acidosis, alveolar ventilation should be improved and the pCO_2 levels should be lowered. Cerebral death will occur from hypoxemia in approximately four minutes if not taken proper care of. Bronchodilators, antibiotics, and physiotherapy can aid in curing the disorder and raising the pH to the normal level of 7.4.

Metabolic Acidosis

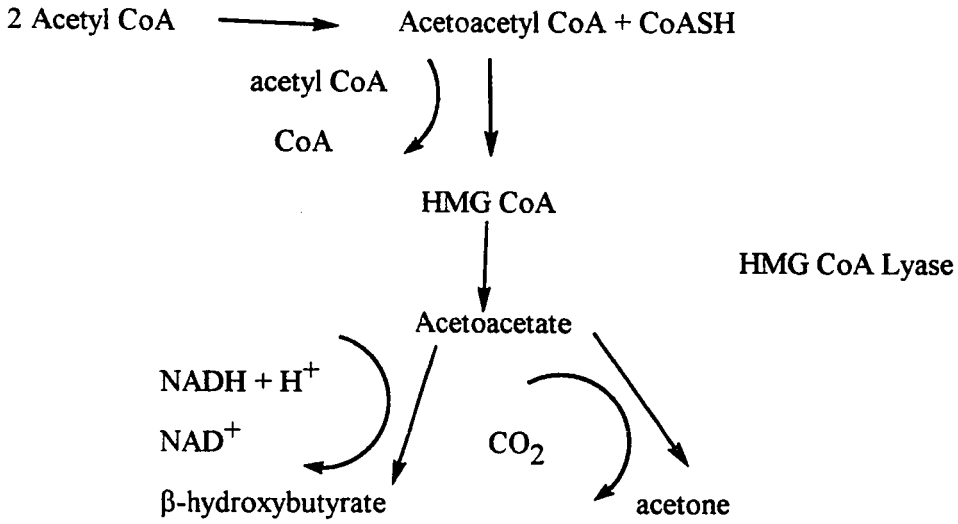
Metabolic acidosis is characterized with a decrease in pH (acidosis) and a decrease in $[HCO_3^-]$ (metabolic). HCO_3^- may be lost because it is excreted directly from the body or because neutralizing acids such as ketoacid, lactic acid, hydrochloric acid, sulfuric acid and phosphoric acid can take the place of HCO_3^- in the blood. (Blick and Liles, 1988). Therefore, the acids are formed at a faster rate than they can be degraded or excreted.

This disorder can occur in two ways: acidemia with a normal anion gap and acidemia with an increased anion gap. The anion gap is the difference between the sum of the measured cations and anions in the plasma or serum calculated as follows: $(Na^+ + K^+) - (Cl^- + HCO_3^-) = < 20$ mmol/L (Stedman's Medical Dictionary, 1990). The cases with a normal anion gap occur when HCO_3^- is lost directly, either in the gastrointestinal tract or the urine. First, when chloride-containing acids (HCl, NH_4Cl) are administered, the anion gap will be normal because the anion, Cl^- is measured with the anions commonly determined in the laboratory (Blick and Liles, 1988). Second, HCO_3^- is lost in the stomach when HCO_3^--containing body fluids (pancreatic secretions) are lost from the

body. This event happens when there is severe diarrhea. Lastly, metabolic acidosis with a normal anion gap occurs in inherited or acquired renal tubular defects such as renal tubular acidosis, which is the failure to acidify the urine despite an acidemia (Blick and Liles, 1988).

When the HCO_3^- in the plasma falls, electrochemical neutrality must be maintained by other anions. When metabolic acidosis occurs, anions such as organic anions, proteins, urates, and phosphates, other than Cl^- and HCO_3^- will exist, but they are not measured in the anion gap. In diseases such as diabetes mellitus, ketoacidosis, lactic aidosis, and also ethanol and methanol poisoning, the anion gap is raised to greater than 20 mmol/L (Marshall, 1992). Diabetes and alcoholism cause an increase in ketoacids. Ketoacids are produced when fats are broken down to acetoacetic acid and β–hydroxybutyric acid. The reaction scheme for the formation of ketoacids from the breakdown of fats is displayed in Scheme 1.

Scheme 1. Breakdown of Fatty Acids to form Ketone Bodies.



Lactic acidosis occurs when there are increased levels of lactic acid in the blood and may take place when there is an inadequate amount of O_2 in the tissues (Blick and Liles, 1988).

Certain diseases have a decreased H^+ excretion from the renal tubules. Renal tubular acidosis, generalized renal failure, and carbonic anhydrase inhibition are some examples of a decreased H^+ excretion (Marshall, 1992).

Compensation for the decrease in H^+ will not occur if the respiratory center is limited. When there is not sufficient O_2 in the tissues as in cases such as hyperventilation, the $[H^+]$ will increase. Therefore, the CO_2 needs to be removed and the $[H^+]$ will decrease, while the pH increases. The management of metabolic acidosis is targeted at reversing of the underlying cause. Thus, the metabolic acidosis patient is given orally a HCO_3^- solution in small aliquots while the arterial $[H^+]$ is measured (Marshall, 1992).

C. Respiratory Alkalosis

Respiratory alkalosis is classified by observing an increased respiratory rate, which leads to an increase in the excretion of pCO_2. Also, the plasma levels of H_2CO_3 and dissolved CO_2 will fall and cause the plasma pH to increase. This disorder is caused by overstimulating of the medulla oblongata and the pons, which are the parts of the brain which control breathing. For example, when a person is anxious, it causes hyperventilation, which occurs when there is too much O_2 entering the lungs (Jackson, 1991). Some of the symptoms associated with respiratory alkalosis are fever, hypocapnia, hysteria, pulmonary embolization, asthma, liver disease, CNS disorders, and primary hyperventilation syndrome (Marshall, 1992; Beeler, 1988).

To compensate for the respiratory alkalosis, there needs to be a reduction in renal H^+ excretion, which will decrease the HCO_3^- in the plasma (Marshall, 1992). Therefore, during compensation, the pH will decrease and revert back to normal.

D. Metabolic Alkalosis

Metabolic alkalosis is caused by an excess of HCO_3^- in the extracellular fluid. This disorder leads to incomplete renal tubular HCO_3^- reabsorption and excretion of HCO_3^- in the urine (Marshall, 1992). Also, reabsorption and excretion usually lasts after the disorder has been corrected. The diseases that cause metabolic alkalosis are: mineralcorticoid excess, Cushing's syndrome (excess Na^+ in the blood), potassium (K^+) depletion, and rapid correction of chronically raised pCO_2 (Marshall,1992).

The causes of metabolic alkalosis can originate in the gastrointestinal tract or in the kidneys. Gastrointestinal metabolic alkalosis usually occurs when the patient tries to treat stomach discomfort by drinking an excess solution of bicarbonate. Also, prolonged vomiting with pyloric obstruction or over-use of gastric suction with loss of stomach contents can occur, which lowers the hydrochloric acid (HCl) levels in the stomach (Zilva and Pannall,1984). The body tends to replace HCl by decreasing CO_2 in the lungs. The retained CO_2 will produce an increase in $[H^+]$ and $[H_2CO_3]$, which will compensate for the alkalosis a slightly (Jackson, 1991). The total combined effect is to replace a strong acid (HCl) by a weak acid (H_2CO_3), although the pH still remains high. Drugs containing sodium bicarbonate, sodium lactate, sodium citrate, and sodium acetate can contribute to metabolic alkalosis because these compounds will react and uptake the H^+ in the stomach.

The renal causes of metabolic alkalosis occur because HCO_3^- is not reabsorbed completely as in normal cases and it is lost in the urine. Therefore, H^+ are also lost in the urine due to stimulation in the extracellular HCO_3^- concentration.

The body's system compensates for this disorder by increasing the pCO_2, which will raise the $[H_2CO_3]$ and then, raise the $[HCO_3^-]$. This process is the body's defense mechanism when the $[H^+]$ gets too low.

In summary, all of these acid-base disturbances cause the body to compensate for either the H_2CO_3, which is the respiratory or acidic component, or the HCO_3^-, which is the metabolic or basic component (Blick and Liles, 1988). These diseases are summarized in Table 4 which shows the effects of each respiratory or metabolic acidosis or alkalosis (Kaplan et al., 1988). The body's way of treating these disorders is to reverse the arrows below, especially the pH.

Table 4. Effects of Respiratory or Metabolic Acidosis and Alkalosis

	pH	pCO_2	HCO_3^-	H_2CO_3
Respiratory Acidosis	↓	↑	NC	↑
Metabolic Acidosis	↓	NC	↓	NC
Respiratory Alkalosis	↑	↓	NC	↓
Metabolic Alkalosis	↑	NC	↑	NC

NC = no change

ANALYSIS OF ANALYTES AND CELLS IN THE BLOOD AND URINE

Analysis of Analytes and Cells in the Urine

In this section, the analytes in the urine will be discussed. In several diagnostic tests, the contents in the urine are a strong indicator that a disease is present. For example, if the pH in the urine is low, there is a possible starvation problem or ketosis. Also, the cells in the urine can show if there is a disease occurring in the body. If there is an increase in the amount of white blood cells in the urine, then the patient would most likely have an infection.

The daily output of urine depends on the fluid intake, the degree of excretion, the salt intake, the temperature, and finally, hormonal control (Kaplan et al., 1988). Urine is formed at a rate of 1 mL/min or 1400 mL $\pm$ 800 mL/24 hours. When the urine is less than the daily output, the condition is called oliguria. Oliguria is seen in victims suffering from shock and poisons. Also, it is seen in pre-renal diseases accompanying low blood pressure, post-renal calculi, and tumors compressing the ureters (Kaplan et al., 1988).

Conversely, when there is an excess amount of urine, it is called polyuria. This condition occurs when there is excretion of large amounts of fluid after excessive salt intake. Diabetics have polyuria when there is an excessive amount of glucose or organic acids filtering in the urine. Polyuria can also be caused from a deficiency of the anti-diuretic hormone (ADH) because this hormone controls the reabsorption of fluids into the body.

In this section, several tests will be described for the analysis of urine. The tests that will be conducted are: pH, glucose, ketone bodies, protein, specific gravity, color, odor, nitrite, leukocytes and blood. Then, a micro examination of the urine will be described to understand the different types of cells found in the urine such as squamous

and epithelial cells, as well as an occasional red blood cell and white blood cell. Microscopes will be used to view the abnormal cells, oval fat bodies, casts, bacteria, parasites, and crystals that are found in the urine of diseased patients.

Test of pH in the Urine

In humans, the physiological range for urine pH is 4.6 to 8.0. The normal pH ranges from 5.5 - 6.5. The urine is seldom alkaline, but the pH is raised in alkalosis after ingestion of alkali from antacids over a period of time. In the cases of an ulcer or a bacterial infection, ammonia is produced (Kaplan et al., 1988). The test for the pH in the urine is commonly completed using a dipstick with two indicators, methyl red and bromthymol blue, which cover the pH range in the body. Then, a color chart is used to determine the pH by comparing the color on the dipstick to the chart.

Test for Glucose in the Urine

This test can be found under the section of Diagnosing Glucose Metabolites in the Blood and Urine (page 21) and the subsection of Tests of Glucose in the Urine.

Test for Ketone Bodies in the Urine

The test for ketone bodies is under the section of Diagnosing Glucose Metabolites in the Blood and Urine (page 21) and the subsection of Test for Ketone Bodies in the Urine.

Test for Protein in the Urine (Proteinuria)

The normal amount of protein filtered into the urine is from 50 to 150 mg/24 hours. Some of the protein is from albumin that is filtered at the glomeruli, but not

reabsorbed in the tubules. Other protein is due to the glycoproteins, which originate from the genitourinary tract lining. In normal urine, the glycoprotein level is usually below 10 mg/dl (Kaplan et al., 1988). When there is too much protein filtering into the urine, the condition is called proteinuria. It usually indicates injury to the glomerular membrane, allowing for escape of protein molecules.

There are two types of proteinuria called transient proteinuria and orthostatic proteinuria. Transient proteinuria results from a high fever, whereas orthostatic proteinuria results when the patient is physically very active (Kaplan et al., 1988). After at least an hour of strenuous exercise, it is normal to observe proteinuria. During exercise, the blood pressure increases which allows more smaller proteins (albumin) to escape into the urine. Proteinuria can be a result of other diseases such as acute renal failure, systemic lupus erythematosis, and the nephrotic syndrome.

One indirect method used to measure the amount of protein in the urine is by using a dipstick. According to Kaplan et al. (1988), it is a "protein error" of indicators, which is a term applied to the change in ionization and pH when an indicator dye is adsorbed in the protein. When there is a shift in pH, it causes a change in color of the indicator. The dipstick is impregnated with a citrate buffer (pH-3.0) and bromphenol blue, which is yellow at pH-3.0 and blue at pH-4.0. At pH 3.0, most protein is in the unionized state. If more protein is present, it becomes ionized causing more dye to ionize until equilibrium is reached. As the urine becomes more saturated with protein, there is more blue color and less yellow color. The dipstick color is compared to the color chart, which ranges from 30 mg/dL to 1000 mg/dL. There is a chance for a false positive with an alkaline urine as in the case of respiratory or metabolic alkalosis (Kaplan et al., 1988).

A second method to detect the concentration of protein uses sulfosalicylic acid. Three milliliters of centrifuged urine are added to a test tube. Then, three drops of 250g/L

sulfosalicylic acid is added slowly down the side of the tube. The acid forms a layer below the urine and it is important to not mix the mixture. After one minute, the turbidity of the urine is examined. There is a grading system of one to four; one contains the lowest amount of protein and four contains the highest amount of protein. False positives will occur if the patient was recently injected with x-ray contrast media, sulfonamides, tolbutamides, or other media (Kaplan et al., 1988).

Test for Blood in the Urine

If small amounts of occult blood (blood cells or hemoglobin) are present in the urine, the blood is usually not colored unless there is enough red blood cells to turn red. The dipstick method is based on an enzymatic reaction of hemoglobin in decomposing peroxides. The dipstick is impregnated with buffered organic peroxide and o-tolidine. If hemoglobin is not present, an orange color is produced. If hemoglobin is present, it produces a blue color within 30 seconds. The end reaction is identical to the reaction of glucose with glucose oxidase (Kaplan et al., 1988).

Hemoglobin is seen in the urine when there is hemolysis in the blood stream or lysis of red blood cells in the urinary tract. This finding is most common in renal diseases or conditions affecting the renal tract. Also, it will be found in the urine among menstruating women. False positives can be due to certain oxidizing contaminants, such as hypochlorite or microbial peroxidases associated with urinary tract infection.

Other dipstick methods can be used for the detection of bilirubin, urobilinogen, nitrite, and leukocyte esterase in the urine, but they will not be discussed because they are beyond the scope of this review (Kaplan et al., 1988).

Urine Concentration Test (Specific Gravity)

An urine concentration test is used to test the tubular function of the kidney. The patient must not drink any fluid or water for 14 hours before the test, eats an early supper and is allowed no food or water after 6 P.M. on the night preceding the test (Kaplan et al., 1988). The urine is discarded during the night and the first specimen in the morning. The second specimen is collected at 8 A.M. and the specific gravity is measured. Usually the specific gravity ranges between 1.010 and 1.030. The one in the numbers before the decimal point represents the water, while the numbers after the decimal point represent the filtered solids (Kaplan et al., 1988). If the patient has a specific gravity of > 1.022, the patient has adequate renal concentrating power. If the values are < 1.022, then another sample is collected at 9 A.M. and assayed. If the concentrating power should fall to zero, then the specific gravity would be 1.010. When a patient has an increased specific gravity, then it indicates dehydration. When there is a large decrease in the specific gravity, then the patient usually has renal problems and a difficult time in concentrating their urine (Kaplan et al., 1988).

Test for Nitrite in the Urine

Normally, no nitrite (NO_2^-) is detectable in the urine. However, it is observed in the urine when there is a urinary tract infection because nitrate (NO_3^-) is reduced to nitrite (NO_2^-). Therefore, a positive test for (NO_2^-) in the urine usually indicates a urinary infection.

Test for Leukocytes in the Urine

The normal specimen shows a generally negative result for leukocytes. When

there are leukocytes in the urine, then there are leukocytes in the urinary tract. Leukocytes are present there when there are invading bacteria. A positive test for leukocytes in the blood indicates a urinary tract infection. Also, positive results may be found in females due to contamination of the specimen by vaginal discharge. A positive urine specimen will show 5-15 cells/hpf in clinical urine.

Color of Urine

The normal color of urine is light straw to dark amber; dark if it is concentrated (more solutes) and light if it is more dilute. Different colors of the urine suggest diseases or drugs. Table 5 shows the color of the urine and what causes the urine to be that color.

Table 5. The Color of the Urine and Its Related Diseases.

Color	**Disease or Cause**
red, pink, pink-brown	fresh red blood or hemoglobin due to bleeding in the genitourinary tract. Also, due to PSP, myoglobin, porphyrins.
brown	methemoglobin, porphyrins, melanin seen in yellow fever. Also, bile pigments can be brown.
black	increased hemoglobin and melanin seen in hemolysis. Also, homogentistic acid excreted in a rare genetic disease called alkaptonuria.
green	due to biliverdin seen in liver diseases, biliary tract disease
yellow-brown	due to bilirubin seen in liver diseases, biliary tract disease
smoky	due to the presence of red blood cells

Odor of Urine

Fresh urine may have the odor of the food eaten. In diabetes mellitus, the odor is a fruity odor caused by ketoacids and acetone. In maple syrup disease, which is due to a rare genetic defect, the urine has the odor of carmelized sugar or maple syrup. When a urine specimen is old, there is an odor of ammonia (NH_3) because the bacteria releases the compound. Also, a putrid odor is present when the urine has undergone bacterial decomposition (Kaplan et al., 1988).

Microexamination of Urine Sediments

These tests are usually not performed on every patient unless the patient is suspected to have renal disease or a positive test for nitrite and leukocyte esterase. A positive test for NO_2^- indicates a bacterial infection and a positive test for leukocyte esterase indicates that there are white blood cells in the urine. For the best concentrated specimen, it is important to examine the urine within the hour. It can be delayed if the urine is refrigerated or .2 mL/100mL of formalin is added. Then, 12 mL of well-mixed urine is centrifuged for five minutes at 80 x g and all but .2-.3 mL is decanted. Then, the urine is suspended by flicking the bottom of the tube on a test tube rack and a drop is used for the micro exam (Kaplan et al., 1988).

The normal urine has squamous and epithelial cells, which have no pathological significance. Normal urinary tracts are continually replacing squamous and epithelial cells just like the skin is always replacing the dead cells. Occasionally, there will be a red or white blood cell. There are some hyaline casts usually after stress, exercise, or fever in the absence of renal disease. Bacteria may be present as external contamination or it will be seen in a kidney or bladder infection (Beeler and Catrou, 1992).

There are many abnormally formed cells found in the urine of diseased patients. If there are red blood cells (RBC's) in the urine, they may originate from any location in the urinary tract. They may also be observed in women's urine during menstruation. The red blood cells found in the men's urine are never normal. A large number of white blood cells indicates an infection in the genitourinary tract (Kaplan et al., 1988). Yeast cells can be found in urine and they are usually recognized by their oval shapes and buddings.

Oval fat bodies are abnormal in the urine and they are usually present to some degree in all diseases affecting the renal parenchyma (cells that compose the glomeruli), but are most present in the nephrotic syndrome. In the urine, the oval fat bodies can have a glowing tint to them under the ultra-violet light. It is postulated that the oval fat bodies are degenerated tubular epithelial cells that have become filled with fat droplets (Kaplan et al., 1988).

Casts are formed by precipitation of mucoprotein in the lumen renal tubules and collecting ducts. Cellular elements are commonly trapped in the casts. One type of cast is RBC cast, which is reddish-brown in color from the hemoglobin leaking out of the broken down red blood cell. These RBC casts always denote a pathological condition such as glomerular inflammation and bleeding. It is associated with glomerulonephritis and systemic lupus erythematosus with kidney involvement (Kaplan et al., 1988). White blood cell casts, embedded with leukocytes, indicate the presence of an infection.

Hyaline casts are glass clear because they have almost the same refractive index as urine. The urine must be fresh, otherwise the bacteria will hydrolyze the hyaline casts. They are present after strenuous exercise, but they can be found in diseases such as proteinuria (Kaplan et al., 1988).

At times, urine sediments will contain crystals. These may be formed from several causes and often have no significance. The crystals that may be found in the urine are:

calcium phosphate ($Ca_3(PO_4)_2$), calcium oxalate (CaC_2O_4) (indicates kidney stones), amorphous phosphate (PO_4^{3-}), sodium urate ($NaC_5H_3N_4O_3$), ammonium urate ($NH_4C_5H_3N_4O_3$), and calcium carbonate ($CaCO_3$).

If a large amount of uric acid crystals are present, they may indicate the breakdown of tissue cells or gout. Gout is an inherited disorder of purine metabolism occurring predominantly in men. It is characterized by an increased but variable uric acid concentration, according to Stedman's Medical Dictionary (1990). Also, gout's symptoms have severe recurrent acute arthritis resulting from deposits of sodium urate crystals in connective tissues and articular cartilage. Increased concentrations of sodium urate crystals are observed in the urine after either a raise in the breakdown of nucleic acids or nucleoproteins in diseases such as leukemia, polycythemia, and toxemia of pregnancy. Decreased concentrations of sodium urate crystals are observed after administration of adrenocorticotropin hormone or cortisol-like steroids. Also, certain drugs decrease the reabsorption of urate by the renal tubules such as allopurinol, aspirin, probenacid, and penicillamine (Kaplan et al., 1988). Patients with gout should avoid eating foods containing high contents of nucleic acids and proteins. Some of these foods are: liver, brain, sweet breads, and some meats.

ANTIGEN AND ANTIBODY REACTIONS

This section contains information about how an antigen reacts with an antibody. The students will conduct an Ouchterlony test to learn about the specificity of the antigen (Ag)-antibody (Ab) reaction. This test is used for isotype identification or immunoglobulin (Ig) identification. Also, the Ouchterlony test is sometimes used for semi-quantitative analysis in human serology. The precipitation titer of an antiserum can be measured when different concentrations of antigen are used in the outer wells (Stites et al., 1987).

The primary interaction of an antigen and an antibody gives rise to a number of secondary events. These are: precipitation, agglutination, phagocytosis, cytolysis, and neutralization (Roitt, 1984). According to Stedman's Medical Dictionary (1990), an antigen is an allergen or an immunogen; any substance that, as a result of coming in contact with appropriate tissues of an animal body, induces a state of sensitivity or resistance to an infection or toxic substance after a latent period (8 to 14 days). An antibody is an immune or protective protein called an immunoglobulin. It is evoked in man or other animals by an antigen and characterized by reacting specifically with that antigen.

The different classes and functions of human immunoglobulins are discussed because of their importance with the ABO and RhD antigens located on the surface of red blood cells. The ABO blood groups are the antigens that are on the surface of the red blood cell membrane and each human has a specific blood group. The blood groups can be AO, BO, AB, and OO on the blood cell. The RhD antigens are also located on the surface of the blood cell membrane. Humans can be either RhD (positive) or Rhd (negative). This RhD antigen is important during pregnancy and during blood transfusions.

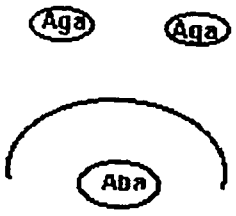
In the ABO blood group, the antigens are IgMs. The sensitized Rh negative mother with a Rh positive child has IgG immunoglobulins cross the placenta and it can be very dangerous for the second pregnancy. The Rh negative mother can have babies with different blood types coming from the father's gene. Therefore, it is important to know the differences between the five human immunoglobulins. Also, the identification of the patient's blood type along with the Rh factor is essential when the patient undergoes a blood transfusion. The blood types must be matched correctly, otherwise a patient could go into shock, acute renal failure, or death. The procedure is known as a cross match.

Ouchterlony Test

A test to visualize the reaction of an antigen with an antibody is called an Ouchterlony Test. The antigen and the antibodies are placed in separate wells cut in agarose gel. They diffuse towards each other and an opaque line will form if there is a specific reaction. This line is caused by the precipitation of antibody and antigen in the optimal proportions. There are three patterns that can result when the test is conducted. First, if there is one antibody (Ab_a) in the center well and two specific antigens (Ag_a) for the antibody (Ab_a) in the peripheral wall as in Diagram 6, then a precipitation will form a solid line, since the Ab_a antibody recognizes both Ag_a antigens. This is called an identity. In Diagram 7, there are two antigens in each well (Ag_{ab}) and (Ag_{ac}). The antibodies (Ab_a) and (Ab_b) will only react completely with the antigen ($Ag_{a,b}$) and partially react with the antigen ($Ag_{a,c}$). There is a longer line to the $Ag_{a,c}$ antigen because that part of the line is the Ab_b antibodies reacting with the $Ag_{a,c}$ antigen. This is called a partial identity or a "spur form" (Roitt, 1984). In Diagram 8, there are two antibodies (Ab_a) and (Ab_c) in the center well. Then, on the outside, there are two antigens (Ag_a) and (Ag_c). The antibodies and antigens only partially react with each other and therefore, there is a

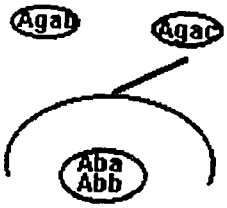
cross because of each antibody only recognizing and reacting to its own antigen. This is called the non-identity form.

Diagram 6. Identity



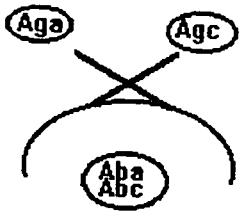
Identity

Diagram 7. Partial Identity



Partial Identity
"spur form"

Diagram 8. Non-Identity



Non-Identity

In humans, there are five structural types or classes of antibodies that can be distinguished from each other. They are called IgG, IgM, IgA, IgD, and IgE. IgG is the most abundant (80%) in extravascular fluids where it combats the microorganisms. These antibodies are able to cross the placenta and provide a major defense against infection for the first couple of weeks post-partum. The baby's immune system is still immature and the mother's antibodies are used to protect the baby's immune system for four to six weeks after birth. IgM is a very effective agglutinator and it is produced early in the immune response. It is the largest immunoglobulin and it comprises 6% of all the immunoglobulins. IgA is the major immunoglobulin in sero-mucous secretions where it defends the external body surface during B-cell development. IgA is 13% of all the immunoglobulins. IgD (0-1%) is almost all completely present on the lymphocyte surface. IgE protects the external body surfaces and recruits anti-microbial agents. Most IgE's are responsible for atopic allergies and only contribute to 0.002% of all immunoglobulins. (Roitt, 1984; Mengal, Frei, and Nachman, 1972).

The Blood Groups

The surface of the red blood cell contains a large number of antigenic determinants that are direct or indirect products of genes (Stites, Stobo, and Wells, 1987). These antigenic determinants constitute different blood group systems. Within each system, antigens seem to be inherited as a product of a single gene or a group of closely linked genes. At least fifteen blood groups have been recognized in man. The ABO blood group, discovered by Landsteiner in 1901, is the dominant system of the human RBC membrane (Roitt, 1984; Roitt et al., 1985). It is the most common used in hospital blood banks. An individual with a particular blood group can recognize the red blood cells carrying different blood group antigens and produce antibodies to them.

The ABO blood groups are very important and most significant in a transfusion because of all the naturally occurring antibodies. Every human has antibodies to either the A factor, B factor, both, or none. If the blood types were not considered during a transfusion, almost half of the North American population would have gross biologic incompatibility and would result in shock, acute renal failure, hemorrhagic syndrome, or death, according to Mendel et al. (1972).

In the ABO system, the antigenic groups are A, B, and H substances. The H substance is an oligosaccharide; the last three sugars in the stem chain are N-acetylglucosamine, galactose, and fucose. The A and B substances are derived from H substances by the action of glycosyl transferases encoded by A and B genes, respectively. These genes produce this enzyme that conjugates the terminal sugars to the H substance. These transferases are specific for their substrates: A transferase for N-acetylgalactosamine and B transferase for galactose. O genes produce no transferase to change the H substance. Therefore, type O persons have only the stem substance, H antigen (Stites et al.,1987). Without the family studies or direct transferase

determinations, it is not possible to distinguish between type A or B homozygous, with two A or B genes, or heterozygous, with one A and one O or one B and one O. Individuals with both A and B genes have two antigens on the red blood cells. People who have no antigens on their red blood cells are type O blood (Roitt, 1984; Roitt et al., 1985; Stites et al., 1987; Mengel et al., 1972).

Individuals with type A blood have anti-B antibodies, while individuals with type B blood have anti-A antibodies. The structure of A and B are drawn below in Diagram 9. In type A and B individuals, N-acetylgalactosamine and galactose are bound to the last galactose in the H substance, respectively in Diagram 9 below (Stites et al., 1987). Also, in type A, B, and O individuals, the fucose molecule is attached to the end galactose on the stem chain of the H substance.

Diagram 9. Structures of the A, B, and H Blood Substances

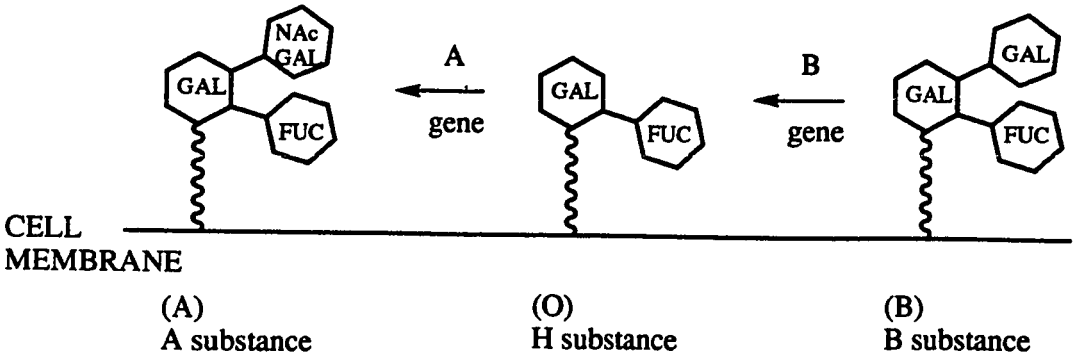


Table 6 below displays the various blood groups and their genotype, antigen, and antibody serum (Roitt, 1985).

Table 6. A, B, O Blood Groups with their Genotype, Antigen, and Antibody Serum

Blood group and frequency	**genotype**	**antigen**	**antibody serum**
A (42%)	AA, AO	A	anti-A
B (8%)	BB, BO	B	anti-B
AB (3%)	AB	A and B	none
O (47%)	O, O	H	anti A, anti B

There are four types of agglutination tests for the ABO blood groups. The tests are: (1) Saline Test, which use red blood cells suspended in saline and antibody serum; (2) Albumin Test, which is similar except the mixture is fortified with bovine serum albumin; (3) Antiglobulin Test, which depends on the rabbit antibody to the human globulins which may have coated cells in the above systems without causing agglutination; and (4) Enzyme Tests, in which trypsin, ficin, or papain are added, which splice the substances at different regions (Mendel, 1972). The Saline Test is adequate when there are naturally occurring antibodies which are gammamacroglobulins (IgM), but the reaction can be potentiated by altering the electrical charges on the cell. Both the Albumin and the Enzyme tests are accurate tests to use and some early IgMs can only be detected by enzymes. The indirect antiglobulin (Coomb's Test) is necessary when the antibody-coated cells need to be detected by either reacting with the complement or the globulin antibodies (Mendel, 1972).

In about 80% of people, soluble blood group antigens appear in the saliva, milk, and other body fluids. A person who is a secretor of their blood group antigens has a Mendelian dominant trait. The secretion of ABH substances is regulated by allelic Se and se genes that are independently inherited from the ABO system (Stites et al., 1987). To detect the blood groups in the saliva, hemagglutination is inhibited after boiling and centrifugation to remove enzymes and other proteins. The blood group substances are found also on cigarettes and sweat spots, which can be used in criminal prosecutions. A compound called phenolthiacabamazine reacts with the soluble blood group antigens, which has a bitter taste when the reaction takes place. If the person tastes nothing at all, then the person is a non-secretor.

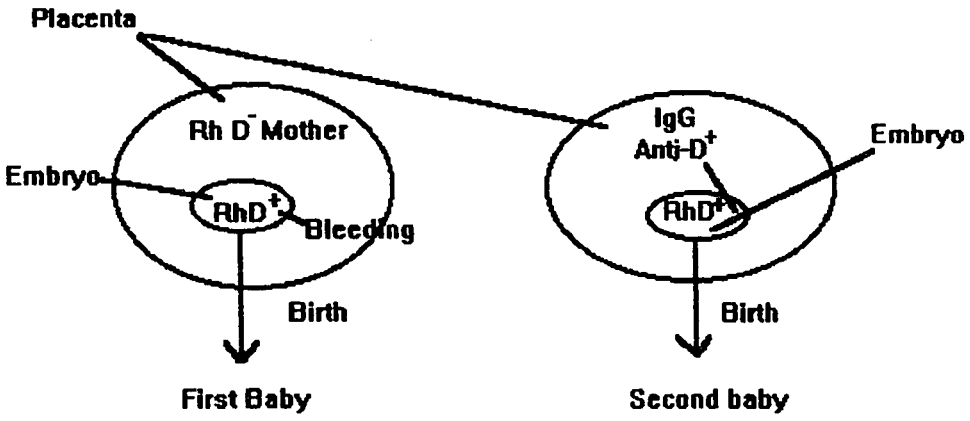
The Rhesus (Rh) blood groups are another dominant antigenic group discovered in 1940 by Landsteiner and Wiener (Stites et al., 1987). It is named because Landsteiner and Weiner observed the immunization of guinea pigs and rabbits with rhesus monkey blood. They obtained an antibody that would agglutinate in about 85% of human adult erythrocytes. The group was named Rh, although the rhesus monkey antigen was later shown to be different from the human RBC antigen. The RhD antigen is determined by three closely linked genes, cDE, where D is the dominant antigen (Stites et al., 1987; Roitt, 1985).

In the North American population, the percent of Rh positive is 85% and Rh negative is 15%. Therefore, 15% of the population is in danger of getting a transfusion from the other 85%. In other populations such as the African American and Chinese populations, there are almost no Rh negative persons, so the Rh problem is essentially nonexistent (Stites et al., 1987).

Hemolytic disease of the newborn, also known as erythroblastosis fetalis, is a disease when specific maternal antibodies react with the fetal red blood cells causing their

premature destruction (Roitt, 1984; Roitt et al., 1985; Mendel et al., 1972; and Stites et al., 1987). It is called erythroblastosis fetalis because before the doctors knew about the Rh factor, the babies developed big red blood cells that would burst. Also, these "Rh babies" had an increased amount of fluid retention, which makes the delivery very difficult. When the red blood cells from the first Rh positive child enter the maternal circulation during pregnancy, the greatest amount passes through during delivery. Consequently, the mother produces anti-Rh antibodies, predominantly IgG, that can then cross the placenta in any subsequent pregnancy. This is the cause of the hemolytic disease of the newborn. Diagram 10 shows what happens in the two different pregnancies.

Diagram 10. Hemolytic Death of the Newborn



These anti-D antibodies fail to agglutinate RhD positive red cells *in vitro* because the low density of the antigenic sites (Roitt, 1984). There are only 10,000-30,000 Rh antigen sites on each red cell compared to 1,000,000 A antigen sites. Thus, only a few antibody molecules can be bound to the cell surface. If the antibodies are IgG class, then the cells are not agglutinated in normal physiologic saline, since there cannot be enough

Rh antibody molecules to counteract the repulsive forces that hold the cells in suspension (Stites et al., 1987; Roitt, 1984).

Agglutination of IgG antibodies can be enhanced by using antiserum to IgG (Coomb's test), or by adding 20-30% bovine serum albumin (BSA) to the reaction mixture, or by treating cells with a proteolytic enzyme such as papain or bromelain (Stites et al., 1987).

A mother with RhD negative blood (dd) can be given a γ–globulin containing antibodies by passive transfer at the time of the greatest placental red blood cell leak, i.e., parturition, then the active antibody production which would affect future pregnancies can be prevented (Mendel et al., 1972). A drug that is now used for this Rh problem is called Rhogam, which is given within 48 hours of the first Rh positive birth. After each incompatible birth, the mother must be given Rhogam again. Its successful use has decreased the number of births of the hemolytic death of the newborn.

DESCRIPTION OF LESSON PLANS

These five lesson plans are designed to facilitate the high school teacher's understanding of chemistry curriculum. On the first page of each lesson plan, there is an outline consisting of the subject, the lesson unit, and the lesson title. Furthermore, the objectives in each module are given to guide the teacher and to focus on specific areas in Clinical Chemistry. Following this introduction, the materials for each lesson plan are listed to indicate what is needed for the lesson as well as where to purchase and price the materials. The first page has a closure, which can be an activity or an assignment to summarize and finish each lesson plan. Also, there is an evaluation section which provides different ways the students will be evaluated throughout each module.

The second page of each lesson plan contains an introduction section outlining the activities and discussions that the teacher will use for the class. An attention getter is the first part of each introduction. The purpose of the attention getter is to attract the students into one or more of the topics in each module, so the students want to do more activities involving each topic. Then, there is either a discussion or activity section for the students to participate in. Some lesson plans have activities involving cooperative groups to enhance group learning with each student having a distinguishable role. An evaluation section at the end of each lesson plan lists the assignments and tests that the students will complete.

In the lesson plans, attention getters, activities, discussions, and evaluations are bolded and italicized. The italicized words are the key words that the teacher emphasizes during the discussion or activity. The underlined words are used to clarify either the homework or specific activities. This format helps the teacher organize the daily classroom routine.

CURRICULAR MATERIALS SECTION

Lesson Plan # 1- Introduction to the Clinical Laboratory

1. dice
2. crackers
3. plastic half-body displaying digestive system

Lesson Plan # 2- Diagnosing Glucose Metabolites in the Urine

1. Ames Keto Diastix (50 per package)- $9.79 at Long's Drugs
2. Ames Glucose Diastix (50 per package)- $5.29 at Long's Drugs
3. Diabetic Glucose Monitor from Lifescan, Inc.
4. Ink Pens
5. Paper Towels

Lesson Plan # 3- The Blood Gases

1. Paper Bags

Lesson Plan # 4- Analysis of Analytes and Cells in the Urine

1. Ames Urinalysis Reagent Strips
   Multistix 8 SG strip (glucose, ketones, specific gravity, blood, pH, protein, nitrite, leukocytes) 100 strips-$50.00
   Available at this address: **Miles Inc.**
   Diagnostic Division
   Elkhart, IN 46515

2. Fleischmenn's Yeast- $1.15 at any supermarket
3. Acetone- hardware store- OSH, Home Depot
4. Glucose- 250 g for $17.20 (Sigma, page 475)
5. Sodium Urate- 5 g $15.95 (Sigma, page 1035)
6. Bovine Serum Albumin (BSA)- 10 mg for $26.20 (Sigma, page 1473)
7. Ammonia- 3 drops of concentrated NH_3 per group
8. Monocular Microscopes- Biology department
9. Biohazard Bags- 1 package- $8.95 (Sigma, page 2211)

Lesson Plan # 5- Antigen and Antibody Reactions

1. Test package- A, B and O blood package- Carolina Biological Supply
2. Filter Package
3. IgG Sheep Serum 16110-017- 100 mL $13.75 (Gibco, page 5-11)

4. IgG Goat Serum 16210-015- 100 mL $19.50 (Gibco, page 5-11)
5. IgG Bovine Calf Serum 16170-029- 100 mL $10.00 (Gibco, page 19-5)
6. IgG Rabbit Serum 16120-016- 100 mL $21.75 (Gibco, page 5-12)
7. Anti-goat Serum Suspended in Agarose-2 mL $41.85 (Sigma, page 1349)
8. Phenylthiacabamazide Test Strips
9. Agargel- 100 g- $16.85 (Sigma, page 1628)
10. Video on Ouchterlony Test

Reagents and equipment can be purchased from:

1) **Sigma Chemical Company**-1995 Biochemicals and Organic Compounds for Diagnostic Reagents

Address: P.O. Box 14508
St. Louis, MO USA 63178-9916
Phone: 1-800-325-3010

2) **Gibco BRL**-1995-1996 Product Catalogue and Reference Guide
Phone: 1-800-828-6686

LESSON PLAN # 1

SUBJECT: CHEMISTRY

LESSON UNIT: Clinical Chemistry

CONCEPT: Clinical chemistry has several relevant applications that the students can use as an introduction to what occurs inside their bodies.

LESSON TITLE: Introduction to the Clinical Laboratory.

OBJECTIVES:

- To give the students an overall introduction to clinical chemistry.
- To learn the measurements and units to understand the analyte concentrations in the clinical laboratory.
- To review the digestion of proteins, fats and carbohydrates throughout the body.

MATERIALS: Dice
Crackers
Plastic half-body model of digestive system

CLOSURE: Tell the students what this unit is composed of and the purpose of the unit and what the students will be doing in the next couple of weeks.

EVALUATION: There will be a quiz on quality control, digestion, and measurement conversions. Also, a worksheet will be given to practice measurement conversions.

INTRODUCTION:

***Attention getter*:**

Patient History Sheet - Distribute to the students. The students will work in pairs and ask each other to fill in the form with a disease and symptoms of the disease.

1. Introduction to Clinical Chemistry

A. Hand out the pretest (see page 74) to determine how much the students know about the different areas that the five lesson plans cover.

B. ***Discussion***

Why is it important to doctors and medical laboratory technicians to understand the meaning and interpretation of the clinical lab results?

1. Definitions of *quality control and quality assurance*
   a. *Accuracy and Precision*
      1. ***Activity***
      - Bull's Eye Demonstration. Either bring in a dart board or draw one on the board and let the students participate in playing a game and deciding whether they were more accurate or precise.
      - Dice. The students play with dice to see how accurate and precise they can be with one or two dice. Will their accuracy or precision improve with more than one dice?

2. Analytical methods
   a. Reliabilty and quality of measuring devices.
   b. Definitions for *sensitive and specific tests*.
   c. Pass out the Biochemical profiles that are used in the hospital.
   d. *Reference Range* of an Analyte
   Use a graph like the one in the chemical literature review to explain the reference range-normal values and abnormal values (see page 15).

3. Different stages of a disease
   A. Defining *Diagnosis, Prognosis, Monitoring, and Screening*

1. ***Activity***- Have students get into groups of three at random and assign them a stage of a disease. Have two students *act* out one stage of a disease to the other students in front of the class.

B. **Oral mini-quiz** of all the disease stages by the teacher who will tell the students a hypothetical story. For example, a woman comes in with a sore throat and the doctor takes a throat culture. He discovers that she has strep throat because of the throat culture test. Therefore, strep throat is his diagnosis.

2. Measurements and Units in the Clinical Laboratory (Review)

A. ***Discussion***

1. Units must be clear to the doctors, laboratory techs, and anyone else concerned with the patient's laboratory tests.
2. Need a # and a unit - mass, volume, or concentration
3. *SI units*- kilograms, meters, and liters
    a. decimals-based on powers of ten
    b. units modified by prefix if the base units are too large or too small.
4. *English units*- pounds, yards, and gallons
5. *Glucose units*- usually mass concentration (mg/dL), but the SI unit for glucose is (mmole/L).

B. Homework Sheet on Conversions (page 75)

3. Digestion of Carbohydrates, Fats and Proteins

A. ***Attention getter:***

Give students saltine crackers and have the students chew them without swallowing. Ask the students to think about the taste while they are chewing the crackers. Ask the students if they think there has been a change in the taste of the cracker. Ask why?

Tell the story about the Canadian Indian and how Dr. Beaumont took care of him. The Indian was shot in the abdominal wall, so Dr. Beaumont experiments on him by putting different types of food in the stomach to see how they digest. He did his direct

research with this method. Eventually, the Indian got tired and ran off.

B. ***Cooperative groups***

Have the students get into 6 number groups and then assign the students an organ or a system: the mouth, the stomach, the small intestines, the liver and the lungs and kidneys. These groups are the "*expert groups*" and the students will learn where and how protein, carbohydrate, and fat digestion takes place in their system. Pass out the handouts on each organ.

C. ***Evaluation***

Each student is expected to make and draw a sandwich of their choice as long as it has protein, fat, and carbohydrate in it and label how each is digested throughout the body.

D. ***Activity***

Give the students a model poster of the digestive system. Have the students in their group make a poster of a person and show the foods being digested in different organs and systems by using color pens and drawing arrows. Individually, the students will make their own chart with the systems going vertically and the proteins, fats and carbohydrates going horizontally. The students will fill in the chart for homework.

5. ***Quiz # 1***-50 points (pages 77-78)

MOUTH

Proteins	Fats	Carbohydrates
----------	----------	**Salivary amylase** is an **enzyme** that breaks down the starches into **disaccharides** such as **maltose**, **glucose**, and smaller units of **starch molecules** called **starch dextrins**. Also, **salivary amylase** protects the teeth and it prevents bacterial build up on the teeth.

STOMACH

Proteins	Fats	Carbohydrates
The proteins are digested into large polypeptides by proteolytic enzymes. **Gastrin** is a hormone that releases **HCl** when the protein is in the stomach. HCl triggers the pepsinogen to activate **pepsin** and it digests the proteins into large polypeptides.	----------	An enzyme, **pancreatic amylase** secreted by the pancreas into stomach, **breaks down** the **carbohydrates** further to **maltose**, **D-glucose**, **sucrose**, **lactose**, **isomaltose** and **α limit dextrins.**

SMALL INTESTINES

Proteins

The hormone, **secretin** releases **bicarbonate** ions, which neutralizes the acidity of the stomach and the pH is 7.5-8.5. The enzymes, **trypsin, chymotrypsin,** and **elastase** cleave polypeptides at the amino acid end and the **carboxypeptidases** cleaves the amino acid end from the carboxyl end. Then, the peptides are hydrolyzed by **peptidases** in the absorptive cells, so only amino acids are released into the portal system.

Fats

The fatty acids are broken down by **lipases** in the small intestines. Also, the liver releases **bile** into the small intestines to **emulsify** the fats.

Carbohydrates

The brush border cells break down the sugars even further to **glucose, fructose** and **galactose.**

BLOOD STREAM AND LYMPHATIC SYSTEM

Proteins

The **amino acids** are released into the **portal blood system** and taken to the **liver**.

Fats

The **small** fatty acids are released into the portal blood system and they are water soluble. They are taken to the liver. The **large fatty acids** are **not water soluble**, so there is a **protein carrier** that takes them to the **lymphatic system**. It circulates throughout the body and dilutes the fatty acids and they are absorbed into the **venous blood system**.

Carbohydrates

The **glucose**, **galactose**, and **fructose** are released into the **portal blood system** and taken to the **liver**.

LIVER

Proteins	Fats	Carbohydrates
Glutamine is transported to the liver and may be hydrolyzed to obtain **ammonium ions**. These ammonium ions can be used by the liver for **urea synthesis**.	The fatty acids undergo a process called **beta-oxidation** and they are **broken down** to CO_2, **energy**, and H_2O.	The glucose proceeds either with **aerobic or anaerobic glycolysis** to produce **energy** CO_2 and H_2O.

LUNGS AND KIDNEYS

Proteins	Fats	Carbohydrates
Urea is **filtered** through the kidneys and excreted into the urine. The carbon skeletons of the amino acids may be used for energy.	The H_2O and CO_2 are exhaled through the lungs for excretion. The kidneys also help for the excretion of H_2O.	The H_2O and CO_2 are exhaled through the lungs and the kidney gets rid of bicarbonate, salt, water, and and urea.

Pretest

Name_____
Period_____

1. Define the word glucose. Name one disease that relates to the levels of glucose in the blood.

2. What is Clinical Chemistry?

3. Name the five immunoglobulins (antibodies) produced by humans?

4. What organ does the digestion of proteins begin with in the human body?

5. What is a buffer?

6. Convert 90 mg/dL glucose into mmol/L of glucose.
Molecular weight of glucose = 180 g/mol.

Homework- Section 1

Measurements of Glucose in the Clinical Laboratory

Name_____
Period_____

1. A patient with diabetes has a blood glucose sugar level of 250 mg/dL. What will the amount be in mmol/L? MW $C_6H_{12}O_6$ = 180 g/mol.

2. A diabetes mellitus patient took a Oral Glucose Tolerance Test (OGTT) and the glucose level was 15.8 mmol/L when the normal value is < 6.7 mmol/L. What is the level of glucose in the patient in mg/dL? What is the normal value of glucose after a OGTT? MW $C_6H_{12}O_6$ = 180 g/mol.

3. One of the symptoms of a patient with acute renal failure is hyperglycemia. He has a glucose value of 8.9 mmol/L. What is his glucose level in mg/dL? MW $C_6H_{12}O_6$ = 180 g/mol.

4. Many people think they have hypoglycemia (low blood sugar) when they have not eaten for awhile. A person being tested has a blood glucose level of 45 mg/dL. Based on your answer above, would this person be hypoglycemic?

5. A 27 year old nurse was admitted into the hospital for hypoglycemia and she was unconscious. Her blood glucose level was taken and it was 1 mM. She was treated with intravenous glucose and admitted to the hospital. What was her blood glucose level in mg/dL? MW $C_6H_{12}O_6$ = 180 g/mol.

Name _____
Date _____
Period_____
Total / 50 points

Quiz # 1

1. In your own words, what is the study of Clinical Chemistry?

_____2. The results that lie in between 2 numbers in which these numbers are expected to occur in most individuals.
_____3. The information regarding the likely outcome of the disease.
_____4. The detection of an early stage of the disease or if a person is predisposed to the disease.
_____5. The ideal analytical method which gives the correct result.
_____6. The confirmation or rejection that the disease is present.
_____7. To look at the natural history or the response of the patient.
_____8. A result that is the same if the test is repeated several times.

a. accuracy
b. precision
c. prognosis
d. diagnosis
e. monitoring
f. screening
g. reference range

9. Name three important facts that the doctor should know about his/her patient before he collects a sample of body fluid.

10. A patient with diabetes mellitus has a blood glucose level of 200 mg/dL. What is the amount in mmol/L? MW $C_6H_{12}O_6$ = 180 g/mol

11. A young woman's glucose level was 50 mg/dL. Would she have hyperglycemia, hypoglycemia, or normal blood sugar? Why?

12. A man has found that he has acute renal failure. His blood glucose level was found to be 16.5 mmol/L. What is this value in mg/dL? MW $C_6H_{12}O_6$ = 180 g/mol.

13. When we did the cracker experiment in class, why when you chewed the cracker for a long time did the taste become sweeter? Explain. What is the important enzyme?

14. Where does protein digestion start?

15. _____ is released by the liver into the small intestines to emulsify the fats.

16. When carbohydrates are broken down to _____ and _____, they are excreted through the body through the _____ and _____.

LESSON PLAN # 2

SUBJECT: CHEMISTRY

LESSON UNIT: Clinical Chemistry

CONCEPT: Clinical chemistry has several relevant applications that the students can use as an introduction to what occurs inside their bodies.

LESSON TITLE: Diagnosing Glucose Metabolites in the Blood and Urine.

OBJECTIVES:

- To perform tests to determine the levels of glucose and ketone bodies in the urine.
- To apply different diseases which correlate with the different levels of glucose and ketone bodies in the blood and urine.
- To go to a company where these medical instruments are made and to visually see what the people in the company do by taking a tour around the facility.
- To visually observe and do paper chromatography and compare it to gel electrophoresis and other separation methods.

MATERIALS: Ink Pens
Paper Towels
Ames Keto Diastix (50) $9.79
Ames Glucose Diastix (50) $5.29
Diabetic Glucose Monitor from Lifescan, Inc.

CLOSURE: The students will have the opportunity to talk with a person who has diabetes mellitus type I. The person will come to class and explain how important it is for diabetics to monitor their blood glucose levels. She will tell them how she obtained the disease and what she has done to make it possible to keep living a normal life. The students will be able to ask her questions about her life and coping with the disease.

EVALUATION: The students will be tested in a variety of ways including patient history cases, comparing two diseases, and the levels of different analytes in those diseases. They will do a group presentation on a disease assigned by the teacher.

INTRODUCTION:

Attention getter:

Give each student a piece of paper towel and several types of black ink pens. Have the students do paper chromatography, so they can make a concrete analogy to how scientists do gel electrophoresis. This example is a "simplified version". It can be an example of how we use different techniques and machines to separate out a specific analyte in a bodily fluid.

1. ***Activity***

A. The students will analyze the urine of a diabetic. The tests they will perform are the *glucose dipstick test* and the *ketone dipstick body test*. The teacher will go over the other tests that need to be done on a patient that might have diabetes mellitus such as an *Oral Glucose Tolerance Test, Glycosylated Hemoglobin Test, 2 Hour Postprandial Test* and *Blood Glucose Fasting Test*. They will need to know what the test tells the doctors and the levels of blood glucose, but they will not have to know the procedure.

2. ***Discussion***

A. Also, the students need to realize that when there is a disease for too high blood glucose levels *(hyperglycemia)*, then there must be one or more for when the blood glucose levels are too low *(hypoglycemia)*. Lecture for a little on both *reactive and fasting hypoglycemia.*

B. The students will do an **oral and written presentation** that they will be doing in groups of four. They will randomly pick a disease for the group to research. The choices are: diabetes mellitus type I, diabetes mellitus type II, reactive hypoglycemia, fasting hypoglycemia, acute renal failure, and nephrotic syndrome. They will have two and a half weeks to research the topic and present it to the class with a visual aid included whether it is a poster, a film clipping, computer program, etc.

3. **Homework**-Pass out the Patient Case Histories (page 82-85) for the students to complete at home.

4. ***Field trip*** to Lifescan, Inc. in Milpitas for a day to look at the facility where they make blood glucose monitors for a diabetic.

E. Have a **guest speaker** who is a diabetic come to the high school to speak about her disease and tell her personal experience with the disease and how she maintains living a normal life with diabetes.

5. ***Evaluation***

a. Students will be evaluated on the glucose and ketone body test results.
b. The group oral and written presentations will be graded based on completeness, preparation, and content.

6. **Quiz # 2** worth 50 points (page 86).

Name_____
Period_____

Patient Case Histories

DIABETES MELLITUS TYPE I (Juvenile)

1. A 14-year-old girl had been well until she started to become really thirsty (polydipsia) and had to urinate a lot (polyuria). She began to lose weight and she could not understand what was wrong with her. She went to the doctor. An arterial blood sample was sent for determination of blood pH. She was also given a urine test. Her level of glucose in the blood was 330 mg/dL and she had a positive test for glucose in the urine. She also tested positive for ketone bodies, both in her blood and her urine. The pH of her blood was 7.05. After a day, the doctor made her come into the office in the morning and take an Oral Glucose Tolerance Test and also a Glycosylated Hemoglobin Test. The values were very high. The OGTT value was 500 mg/dL and the glycosylated Hb was 14%. The doctor diagnosed that the girl has Juvenile Diabetes Mellitus Type I (IDDM), which is an autoimmune disorder where the body's antibodies kill the cells in the pancreas that produce insulin. Therefore, the Type I diabetics are insulin-dependent; in another words, they need insulin to live. This disease is either genetic or environmental and the onset of IDDM is usually in childhood.

1. What are the symptoms of IDDM?

2. What were the tests given in order to diagnose that the girl has IDDM?

3. What were the results of the test and what are the normal values?

4. What is the cause of IDDM?

5. When is IDDM usually diagnosed?

DIABETES MELLITUS TYPE II (Adult-onset)

2. A 40-year-old woman was seen in a diabetes outpatient clinic. Her chief complaints were fatigue, increased appetite, thirst, and frequent urination. She tired easily while doing simple tasks around the house. After her noon meal, she always felt tired and had to take a nap. Her symptoms continued and her appetite became excessive. She had to urinate 3 or 4 times during the night and always complained of thirst.

Her family history showed her grandfather had Juvenile Diabetes Mellitus and died of a myocardial infarction (heart attack) when he was 50. Her sister had been diagnosed with IDDM when she was 17.

The patient's physical examination was normal except the patient was extremely overweight. Her blood glucose level was 250 mg/dL and she had glucose in her urine. The glycosylated Hb was 12%. The urine also showed a moderate amount of ketone bodies as determined by an Acetest tablet. Her serum insulin level was within the normal range. The patient had an OGTT test and the level was 300 mg/dL.

The patient has the disease called Adult-Onset Diabetes Type II (NIDDM). This type of diabetes is not insulin-dependent because the problem is a reduced number of insulin receptor sites on the cells that let insulin inside. Therefore, the patient becomes hyperglycemic. It is a strong familial inheritance.

NIDDM can be treatable with a change in diet, weight reduction, and increased physical activity.

1. What are the causes of NIDDM?

2. What are the tests used to diagnose adult-onset diabetes mellitus?

3. What are the symptoms of NIDDM?

4. Do patients with NIDDM have control over treating their disease?

5. What is the difference between IDDM and NIDDM?

REACTIVE HYPOGLYCEMIA

3. A 54-year-old man has Adult-Onset Diabetes Mellitus and has been having dizzy spells of extreme anxiety with sweating, shaking, and confusion on arising each morning. These spells lasted about 10 min and they were relieved by eating breakfast. These symptoms have been occurring for about 6 months, during which he gained 20 pounds. He tends to drink a couple of beers with lunch and dinner and the sweating starts to occur again. After a soda or a meal, he is relieved.

He is taking some drugs to treat the hyperglycemia that occurs with the Adult-Onset Diabetes Mellitus. When he overindulges in alcohol along with the drugs to treat hyperglycemia, it can cause reactive drug-induced hypoglycemia.

This type of hypoglycemia is treated by a change in the diet and psychological support. The patient's blood glucose level after fasting for 48 hours was 50 mg/dL. Otherwise, the physical exam was normal.

1. What are the symptoms of reactive hypoglycemia?

2. What substance can increase the effect of hypoglycemia?

3. How can the patient treat this disease?

FASTING HYPOGLYCEMIA

4. A 70-year-old man was admitted to the hospital for a 72 hour fast. He has an insulinoma tumor, which produces and releases excessive amounts of insulin to the body. Insulin is responsible for lowering the blood glucose levels. Therefore, he develops severe symptoms.

Blood samples were collected every 5 hours and the C-peptide concentration was measured. C-peptide is released from the pancreas in equimolar quantities with insulin. In normal individuals, hypoglycemia suppresses endogenous insulin and C-peptide secretion. The patient's C-peptide concentration was 3.0 μg/L. This high level indicates that endogenous insulin is being released in the body.

When the C-peptide concentration is greater than normal, the patient has fasting hypoglycemia. A 2 hour postprandial (after a meal) test was done and the blood glucose was normal until after 5 hours where it was lowered to 50 mg/dL. His symptoms were a excessive hunger, sweat, anxiety, detachment, and weakness.

1. What are the differences between reactive and fasting hypoglycemia?

2. What is a C-peptide? What was the level in fasting hypoglycemia?

3. What happens to a person with an insulinoma tumor?

Name_____
Date _____
Period_____
Total / 50 points

QUIZ # 2

1. What is the normal reference range of blood glucose in the blood?

_____

2. An increase in the amount of blood glucose is called _____.

3. If a person has an insulinoma tumor, what type of disease/symptom will result?
   a. diabetes mellitus type I
   b. diabetes mellitus type II
   c. reactive hypoglycemia
   d. fasting hypoglycemia

4. On your trip to Lifescan, Inc., what was the most interesting thing you learned?

5. Who do doctors do a glycosylated hemoglobin test for? What does the test tell the doctors? What is the normal range expressed as a percent for the glycosylated Hb?

6. A 13-year-old boy had been well until he realized that he was losing weight and was constantly thirsty. He also had to urinate constantly. The doctor performed several tests for him and his blood glucose level was 150 mg/dL. Also, the urine test showed positive for glucose. He also tested positive for ketone bodies in his urine and blood. The pH of his blood was about 7.10.

a. With what disease would you diagnose this patient? What are some of the key indications of why you chose this answer. Explain.

b. How would you treat the boy's disease?

7. A 60-year-old woman who has Adult-Onset Diabetes Mellitus has been having dizzy spells in addition to sweating and shaking. These symptoms happen the most when she has not eaten for awhile. As soon as she eats a meal, her symptoms are relieved and she feels fine. She takes drugs to treat her hyperglycemia, which can react with alcohol if she drinks too much.

a. What disease does this woman have?

b. How can this disease be treated?

8. What is the cause of insulin-dependent diabetes mellitus (juvenile diabetes)?

9. A _____ test measures the amount of endogenous insulin in the body.

10. Put either an arrow up if the level is increased, an arrow down if the level is decreased, and an arrow across if the level does not change.

	Diabetes Mellitus	Fasting Hypoglycemia
OGTT (glucose)	_____	_____
2 hour post prandial test	_____	_____
pH	_____	_____
ketone body concentration	_____	_____
glucose in urine	_____	_____
C-peptide test	_____	_____

LESSON PLAN # 3

SUBJECT: CHEMISTRY

LESSON UNIT: Clinical Chemistry

CONCEPT: Clinical chemistry has several applications that the students can use as an introduction to what occurs inside their bodies.

LESSON TITLE: The Blood Gases.

OBJECTIVES:

- To introduce the carbonic acid-bicarbonate buffer pair to the students.
- To show how the concept equilibrium works in the body with the buffer.
- What happens when there is an unequilibrium balance of the blood gases and the diseases that result such as respiratory acidosis, respiratory alkalosis, and metabolic acidosis, and metabolic alkalosis.

MATERIALS: Paper Bags

CLOSURE: Mini-quiz- Have the students hold their breath as long as they can. Then, the students will observe and think about what is going on with their bodies. Then, when they are finished, the students will write down what disease they would get if they continued doing this and will explain it using the carbonic acid buffer. Then, the students will grade each other's paper using a rubric scale (5-highest -1-lowest).

EVALUATION: The students will be tested on their knowledge of the carbonic acid buffer. Gamblegrams will be used to display the differences and the students will guess the diseases based on the readings of the different blood gases.

INTRODUCTION:

Attention getter:

Have the students do an exercise involving running up and down the stairs for a minute or longer. After they finish this exercise, they will time how long it takes to have their heart beat return to normal. Also, they will observe what their bodies are doing in response to this exercise. Then, they will repeat the exercise and hold a paper bag over their mouth. They will breathe into the bag and see how much time it takes for their breathing to return to normal. Does it take longer or shorter for their breathing to return to normal with the paper bag?

1. ***Discussion***

Introduce the *carbonic acid buffer* assuming the students have already been taught what a buffer is in relation to acids and bases. This is the most important buffer in the body. It is in *equilibrium* and the most abundant is the HCO_3^-.

2. ***Cooperative groups***

A. Have the students get into groups of 4 by the list in alphabetical order. Assign each person in the group to a disease - respiratory acidosis, respiratory alkalosis, metabolic acidosis, metabolic alkalosis. Have each group research:

1. the cause of the disease according to the buffer equation, example; respiratory alkalosis - increase of CO_2 in the body.
2. how the body compensates for the disease.
3. the diseases where one of these may be a symptom, example; diabetes - metabolic acidosis.
4. the symptoms of the disease.

Then, the students will go back to their other groups and explain their disease and everything about the disease. They will be required to make a chart of the ions and arrows of either up, down, or no change to indicate the differences between the diseases.

3. ***Activity***

A. Pass out the gamblegrams and lecture how to use and read these charts for interpretation. The students will be given a worksheet of gamblegrams to practice their analysis of the electrolytes and

deciding what disease the gamblegram represents. The disease will depend on the amounts of analytes in a patient's plasma sample.

4. ***Evaluation***

A. Students will turn in their gamblegrams and their research on one of the four symptoms.

5. **Quiz #3** will be worth 50 points (page 92-93).

Name_____
Date_____
Period_____
Total / 50 points

Quiz # 3

1. In your own words, define a buffer.

2. What is the most important buffer in our body? _____

3. Write the equation of the buffer in #2. _____

4. A(n) _____ is a mixture of a weak acid and the salt of a base (a conjugate base pair).

5. What equation would you use to calculate the pH of the carbonic acid buffer?

_____

6. What organ helps get rid of the excess hydrogen ions that are produced in the body?

7. When the CO_2 levels are raised or lowered, it results in _____ and _____, respectively.
   a. respiratory alkalosis and respiratory acidosis
   b. metabolic alkalosis and metabolic acidosis
   c. respiratory acidosis and respiratory alkalosis
   d. metabolic acidosis and metabolic alkalosis

8. When the HCO_3^- levels are raised and lowered, it results in _____ and _____, respectively.
   a. respiratory alkalosis and respiratory acidosis
   b. metabolic alkalosis and metabolic acidosis
   c. respiratory acidosis and respiratory alkalosis
   d. metabolic acidosis and metabolic alkalosis

9. When there is not enough O_2 getting to the lungs, what disorder results (ie. respiratory alkalosis)? _____

10. Metabolic acidosis is characterized by a decrease / increase in pH and a decrease / increase in HCO_3^- concentration. Circle the correct answer.

11. What disorder has an increase in organic acids?
    a. respiratory acidosis
    b. metabolic acidosis
    c. metabolic alkalosis
    d. respiratory alkalosis

12. What is the cause of respiratory alkalosis?

13. Prolonged vomiting will lower the _____ levels in the stomach and the body tends to compensate these levels by decreasing the _____ in the lungs.

14. Fill in the following by indicating an increase with an upward arrow, a decrease with a downward arrow, and no change with a "NC".

	pH	H_2CO_3	pCO_2	HCO_3^-
Respiratory acidosis				NC
Metabolic acidosis		NC		
Respiratory alkalosis				NC

LESSON PLAN # 4

SUBJECT: CHEMISTRY

LESSON UNIT: Clinical Chemistry

CONCEPT: Clinical chemistry has several relevant applications that the students can use as an introduction to what occurs inside their bodies.

LESSON TITLE: Analysis of Analytes and Cells in the Urine.

OBJECTIVES:

- To analyze normal and abnormal urine for its contents.
- When the students look for certain analytes in the urine, they will be able to relate these analyte concentrations in association to different diseases.

MATERIALS: Multistix 8SG Strip (glucose, ketones, specific gravity, blood, pH, protein, nitrite, leukocytes) 100 strips- $50.00
Fleischmenn's Yeast - $1.15
Acetone- 1 mL per group
Glucose - 250 g for $17.20
Sodium Urate - 5g for $15.95
Bovine Serum Albumin (BSA) - 10 mg for $26.20
Ammonia-3 drops of conc. NH_3 per group
Monocular Microscopes
Biohazard Bags-1 package- $8.95

CLOSURE: Have the students pick partners and pick a disease. Have them decide what analytes will be present in the urine. Go in front of the class and play out the disease and have the other students guess the disease and write it down. The teacher will have a list of the diseases.

diabetes	acute renal failure
hyperglycemia	systemic lupus erythematosis
hypoglycemia	dehydration
proteinuria	starvation
nephrotic syndrome	gout
maple syrup disease	leukemia
toxemia of pregnancy	polycythemia

EVALUATION: The students will be evaluated on the analytes in the urine. Each student has specific analytes in the test tube. Also, the students will draw the sediments that are found in the urine during microexamination. Then, the student will write what possible diseases the patient has based on the examination of urine.

INTRODUCTION:

***Demonstration*:**

The teacher has a test tube of a diabetic's urine and performs several tests on the urine such as the *Multistix dipstick test*. Then, the teacher writes the tests on the board and which tests turned out to be positive. Also, he/she has some volunteers to come up to smell the urine and to look at the color. The students are now supposed to guess based on the past labs (lesson plan #2) what disease the patient has based on the urine analysis tests. The teacher will write acceptable answers on the board.

1. ***Discussion and Activity***

A. Give the students a brief overview of the output of urine and the analytes found in the urine (see literature review). Explain that the students will be doing *qualitative tests* to see what analytes are in the urine. These are standard known solutions. The teacher will assign the students a date to bring in their urine from that day in a closed container.

1. pH urine (5.5-6.5)
   acidic: diabetes, ketosis, and starvation
   basic: after ingestion of alkali

2. glucose dipstick
   positive: diabetes, hyperglycemia, acute renal failure
   nephrotic syndrome

3. ketone body dipstick
   positive: diabetes, ketosis, and starvation

4. protein dipstick
   positive: proteinuria (transient and orthostatic),
   acute renal failure, nephrotic syndrome,
   systemic lupus erythematosus

5. blood
   positive for hemoglobin: blue color, hemolysis in the blood stream, lysis of RBC in the urinary tract
   renal disease

6. specific gravity 1.010-1.030
   increased: dehydration, starvation
   decreased: renal problems

7. nitrite
   positive: urinary tract infection

8. leukocyte esterase
   positive: urinary tract infection

9. color of urine: light straw to dark amber
   red - RBC or Hb, PSP, myoglobin, porphyrins,
   brown- met Hb, porphyrins, melanin, bile pigments
   black- increased Hb and melanin, homogentistic acid
   green- biliverdin
   yellow-brown- bilirubin
   smoky- RBC

10. odor
   sweet, fruity- diabetes (ketoacids and acetone)
   maple syrup- maple syrup disease
   ammonia- old urine
   putrid- bacterial decomposition

B. **Microexamination**

Have the students *draw* what they see under the microscope and *label* what they see. The urine will be centrifuged and one drop will be placed on a slide. The students will look for normal sediments found in the urine and then the teacher will put some blood and yeast in to show the students what is abnormal.

normal: squamous cells, epithelial cells, occasional RBCs
   WBCs, and casts

abnormal: RBC (hemolysis)
   WBC (infection in genitourinary tract)
   crystals-urate (gout)
   yeast cells-infection

2. ***Activity***

A. The students will bring a sample of their **own urine** to the class and they will perform all the qualitative tests and write their observations down on paper. After all the students gather their information, they will write the results on the board. Then, the class will analyze the data in a *statistical* way. Biohazard bags will be needed to discard the student's urine.

3. ***Evaluation***

A. Each student will be given an unknown solution and they each have to perform all the qualitative tests and the microexamination on the solution. Once they have found out what is in their test tube of urine, the students are required to write down what the possible diseases are by examining the urine.

B. Have the students compare their analyses to other students, thereby confirming the individual's analysis.

Quiz #4 Unknown Solution

Qualitative Tests on the Analysis of Urine Name_____

Test	Standard	Your urine	Unknown
1. pH			
2. glucose			
3. ketone bodies			
4. protein			
5. blood			
6. specific gravity			

7. nitrite

	Standard	Your urine	Unknown
8. leukocytes			
9. color			
10. odor			

<u>Microexamination</u>

Draw what you see and label the sediments by their names. Write a (N) by the sediment if it is normal in the urine and an (A) if it is abnormal.

LESSON PLAN # 5

SUBJECT: CHEMISTRY

LESSON UNIT: Clinical Chemistry

CONCEPT: Clinical chemistry has several relevant applications that the students can use as an introduction to what occurs inside their bodies.

LESSON TITLE: Antigen and Antibody Reactions.

OBJECTIVES:

- To visually observe an antigen and antibody interaction using the Ouchterlony test.
- To visually observe a demonstration of A, B, and O blood typing and the concept of agglutination.
- The students will be able to determine whether they are a secretor.

MATERIALS: Test package- A, B, and O blood package
Filter package
IgG Sheep Serum 16110-017- 100 mL $14.75
IgG Bovine Calf Serum 16170-029- 100 mL $10.00
IgG Goat Serum 16210-015- 100 mL $19.50
IgG Rabbit Serum 16120-016- 100 mL $21.75
Anti-Goat Serum Suspended in Agarose- 2 mL $41.85
Phenolthiacabamazine Test Strips
Agargel- 100 g- $16.85
Video on Ouchterlony Test

CLOSURE: Ask the students to answer this question. Suppose you have an Ouchterlony Test and all the serum IgG such as bovine, goat, sheep, and human react with the anti-serum. The only one that does not react is the chicken IgG. Why? Extra credit points if the student gets the right answer.

EVALUATION: Give the students a preprepared test strip of the A, B, O blood groups with the Rh factor and have the students write the blood type and if the patient is Rh positive. Then, the students will be given different Ouchterlony tests and they will explain why it is either identity, partial identity, or non-identity.

INTRODUCTION

Attention getter:

Have the students taste the test strip paper with the compound *phenolthiacabamazine* to determine whether the student is a secretor of his/her blood group antigens. It will have a very bitter taste if the student is a secretor and approximately 80% of the students will be secretors. Take a vote and see if the numbers of secretors correlate with the statistic.

1. ***Discussion***

   A. Explain the different *blood group antigens (A, B, H)* along with the chemistry of how the different substances are formed. Show the students how they can find out the possibilities of their blood type by their parent's blood type.

   B. Lecture on the significance of the Rh factor and the importance of testing women when they are pregnant. Pass out an article of the discovery of the *Rh factor* on how it affected the population at that time.

2. ***Demonstration***

   A. Show the students how they proceed in testing their *blood type* and *Rh factor*. The teacher will do a demonstration because blood testing is not allowed in the high school classroom. Bring in other results to show other blood types.

3. ***Activity***

   A. Have the students see a video on the *Ouchterlony tests*. The students will make their own Ouchterlony tests with the IgG serums and the anti-goat serum. They will analyze if the test is an *identity*, *partial-identity*, or *non-identity* and understand why some serums react, partially react and others do not react.

4. ***Evaluation***

   A. The students will make Ouchterlony Tests with different serums to learn how the serums and anti-serums react together.

5. **Quiz** on the blood group antigens, Rh factor, and Ouchterlony Tests, page 103-105.

Name _____
Date _____
Period _____
Total /50 points

Quiz #5

1. The ABO blood group antigens and RhD antigens are located on the _____ of the red blood cell.

The following 7 questions are multiple choice. Choose only one answer.

2. This type of immunoglobulin is small enough to cross the placenta.
3. This antibody is produced early in the immune response.
4. The major immunoglobulin in sero-mucous secretions.

a. IgM
b. IgA
c. IgG
d. IgE
e. IgD

5. This type of antibody is almost always present on the lymphocyte surface.

6. This type of antibody is responsible for atopic allergies and only contributes to 0.002% of all the antibodies.

7. This antibody is the most dominant in the ABO blood group.

8. This type of antibody is the most abundant of the five classes.

---

9. Who discovered the ABO blood groups?
   a. Watson and Crick
   b. Landsteiner
   c. Boyle
   d. Einstein

10. Draw the Ouchterlony results and identify which pattern will occur.
The outer wells are antigens and the inner wells are antibodies.

a. AgB AgA

AbA
AbB

b. AgA AgA

AbA

c. AgA AgA
AgB AgC

AbA
AbC

d. AgA AgB
AgA

AbA
AbB

11. Circle either the A, B, or O blood type which has no blood antigens on the surface of the red blood cell.

12. If a person has type B blood, what type of antibodies will this person produce?
    a. anti-A antibodies
    b. anti-B antibodies
    c. anti-AB antibodies
    d. no antibodies

13. What is the genotype for type A blood?
    a. AA, AA
    b. AA, BB
    c. AA, OO
    d. AA, AO

14. What is the antigen for type O blood?

a. A
b. B
c. H
d. O

15. What is hemolytic disease of the newborn? Explain how this disease comes about. (Hint: Rh factor)

16. When is it important to give a pregnant mother a shot of Rhogam? After which pregnancy?

DISCUSSION

This project is written to develop curricula in Clinical Chemistry for the high school Advanced Placement students. After obtaining a concrete background in general chemistry, the students have the opportunity to expand their knowledge into a different realm of chemistry integrated with biology and physiology.

There are five sections which compose this project. Of these five sections, there are five lesson plans or modules, which are designed for the student to comprehend a specific area in clinical chemistry. These lesson plans are most beneficial towards the end of the year since they use underlying concepts which are taught in chemistry class such as units, conversion factors, and buffers.

The educational literature review states that over 50% of the high school students learn at a concrete reasoning level, in which they can relate through direct experience by using the senses. Therefore, each of these modules is designed for the students to perform "hands-on" laboratory experiences. For example, in the *Antigen and Antibody Reactions* lesson plan, the students pipet different types of antigen or antibody into the wells of the agar gel to visualize whether an opaque precipitate forms. By doing the experiment and seeing the results, the students can relate more to how antigens and antibodies react in our bodies. These reactions cannot be visually seen, so the students have to think using their formal reasoning skills. If students are presented with concrete level tasks to represent formal level concepts, the transition to formal cognitive thought can be facilitated and thus, the concepts are more comprehensive.

There are many ways to assess whether the students are learning the clinical concepts involved in these modules. First of all, a pre-test will be given at the beginning of the lesson plans to obtain an idea of the student's knowledge in the area of clinical

chemistry. Each question will be a sample of a topic covered in each of the five modules. Therefore, the teacher has an idea of where to start for an introduction.

After each module, a test will follow different modes of testing. Most of the tests consist of multiple choice, short answer, or fill-in questions. The module test in the lesson plan *Analysis of Analytes and Cells in the Urine* is based on the student's performance in the laboratory. The student will be given an unknown urine sample and perform a series of tests to determine the correct answer.

In addition to tests, assessment will be based on group participation when the students work together cooperatively. The students also will be actively involved in skits and role playing. Oral and written reports will be assigned. All these assessments demonstrate active learning. Also, the students learn important skills in teamworking where each student has an integral role in the learning process.

In conclusion, the goal of this thesis is to implement this curricula into the high school classroom. Therefore, it would be advantageous to use these lesson plans in a high school as a trial run to verify what topics work and which lesson plans need some adjustments. This trial would provide insight in how the students learn these concepts and understand how they apply clinical chemistry to their own bodies. The students will then write their opinions on topics and activities that they liked and disliked. From their evaluations, students can help improve the content of the lesson plans and contribute to creating more lesson plans in clinical chemistry based on their ideas.

Glossary of Terms

agglutination- the process by which suspended bacteria, cells, or other particles of similar size are caused to adhere and form into clumps; similar to precipitation, but the particles are larger and are in suspension rather than being in solution.

albumin- a type of simple protein; varieties of which are widely distributed throughout the tissues and fluids of plants and animals.

aldimine- an aldose group combining with an amine group.

alkaptonuria- excretion of homogentistic acid in the urine due to congenital lack of the enzyme homogentisate 1,2-dioxygenase, which mediates an essential step in the catabolism of phenylalanine and tyrosine. Homogentistic acid is 2,5-dihydroxyphenyl acetic acid.

Amadori Rearrangement- conversion of N-glycosides of aldoses to N-glycosides of the corresponding ketoses by acid or base catalysis.

antibody- immune or protective protein; originally, a body or substance evoked in man or other animals by an antigen, and characterized by reacting specifically with the antigen in some demonstrable way.

antidiuretic hormone (ADH)- a hormone secreted from the pituitary gland that signals secondary hormones to reduce the output of urine.

atopic- relating to atopy, which is a type I allergic reaction, specifically with strong familial tendencies, caused by various allergens and associated with IgE antibody.

autoimmune- arising from and directed against the individual's own tissues, as in autoimmune disease.

β-adrenergenic agent- drug that mimics the actions of the sympathetic nervous system and competes with the β-adrenergenic agonists for available receptor sites.

basal- situated nearer the base of a pyramid-shaped organ in relation to a specific reference point.

bilirubin- a red bile pigment found as sodium bilirubinate, or as insoluble calcium salt in gallstones, formed from hemoglobin during normal and abnormal destruction of erythrocytes by the reticuloendothelial system.

biliverdin- dehydrobilirubin; a green bile pigment formed from oxidation of bilirubin.

botulism- an intoxication due to the ingestion of *Clostridium botulinum* toxin in improperly canned or preserved food and is characterized by paralysis in all species.

calculi- stone, a concentration formed in any part of the body, most commonly in the passages of the biliary and urinary tracts; usually salts of organic and inorganic acids.

chromogen- a substance, itself without definite color, that may be transformed into a pigment; denoting especially benzene and its homologs of toluene.

cytolysis- the dissolution of a cell.

emphysema- condition of the lung characterized by an increase beyond normal in the size of air spaces distal to the terminal bronchiole with destructive changes in their walls and reduction in their number.

etiology- the science and study of the causes of the disease and their mode of operation.

glomerulus- a tuft formed of capillary loops at the beginning of each uriniferous tubule of the kidney; this tuft with its Bowman's capsule constitutes the corpusculum renis (malpighian body).

glucagon- a hormone that elevates the blood glucose levels in the tissue by mobilization of hepatic glycogen.

glycolysis aerobic- the energy-yielding conversion of glucose to pyruvate oxidation products.

glycoprotein- one of a group of protein-carbohydrate compounds among which the most important are the mucins, mucoid, and amyloid.

glycosuria- urinary excretion of carbohydrates; glucose in the urine.

glycosylated- formation of linkages with glycosyl groups, as between glucose and hemoglobin chain to form the fraction HBA_{1c}.

hepatitis- inflammation of the liver; usually a viral infection, but sometimes toxic reagents.

histocompatibility- a state of immunologic similarity or identity of tissues sufficient to permit successful homograft transplantation. Test system for the HLA antigens.

hypertriglyceridemia- elevated triglyceride concentration in the blood.

insulin- a hormone that lowers the blood glucose levels in the tissue.

ketoamine- a ketone group combining with an amine group.

ketonuria- enhanced urinary excretion of ketone bodies.

Kreb's cycle- the sequence of chemical reactions, occurring in the liver, which results in the production of urea.

leukemia- progressive proliferation of abnormal leukocytes found in hemopoietic tissues and other organs, and usually in the blood in increased numbers.

melanin- any of the dark brown to black polymers of indole 5,6 quinone that normally occurs in the hair, skin, pigmented coat of the retina, and inconstantly in the medulla and the zona reticularis of the adrenal gland.

metabolic acidosis- decreased pH and bicarbonate concentration in the body fluids caused either by accumulation of acids or by abnormal losses of fixed base from the body, as in diarrhea or renal disease.

mucoprotein- general term for protein-polysaccharide complex, usually implying that the protein component is the major part of the complex.

nephrotic syndrome- a clinical state characterized by edema, albuminuria, decreased plasma albumin, and usually an increased blood cholesterol level; lipid droplets may be found in the cells of the renal tubules, but the basic lesion is increased permeability of the glomerular capillary basement membrane.

occult- hidden; concealed. Denoting a concealed hemorrhage, the blood being so changed as not to be readily recognized.

pancreatic islets of Langerhans- cellular masses varying from a few to hundreds of cells lying in the interstitial tissue of the pancreas. Place where insulin and glucagon are produced.

parenchyma- the distinguishing or specific cells of a gland or organ, contained in and supported by the connective tissue framework or stroma.

phagocytosis- the process of ingestion and digestion by cells of solid substances, e.g. other cells, bacteria, bits of necrosed tissue, foreign particles.

phenolsulfonphthalein (PSP)- phenol red; occurs as a bright dark red crystalline powder; widely used as an indicator in tissue culture media.

plasma- blood; the fluid (non-cellular) portion of the circulating blood, as distinguished from the serum obtained after coagulation.

poliomyelitis- inflammation of the gray matter of the spinal cord marked by fever, pains, and muscular atrophy.

polydipsia- excessive thirst.

polycythemia- an increase above normal in the number of red blood cells in the blood.

polyuria- excessive urine.

porphyrins- pigments widely distributed throughout nature (heme, bile pigments, cytochromes) consisting of four pyrroles joined in a ring (porphyrin structure).

postgastrectomy- following excision of a part or all of the stomach.

postprandial- after a meal.

Schiff base- any compound with a carbon-nitrogen double bond; organic chemists call them imines.

sero-mucous secretions- pertaining to a mixture of watery and mucinous material, such as that of certain glands.

sensitization- immunization, especially with reference to antigens not associated with infection; the induction of acquired sensitivity or of allergy.

serum- the fluid portion of the blood obtained after the removal of the fibrin clot and blood cells, distinguished from the plasma in circulating blood.

S.I. units- the current system of measurements called the Systeme International d'Unites was accepted internationally in 1960.

sulfonamides- the sulfa drugs, a group of bacteriostatic drugs containing the sulfonamide group.

sulfonylureas-derivatives of isopropylthiodiazylsulfanilamide, which possess hypoglycemic action.

systemic lupus erythematosus- an inflammatory connective tissue disease with variable features including fever, weakness, and fatigability, joint pains, or arthritis, skin lesions on the face, neck and upper extremities, anemia, glomerular lesions, hyperglobulinemia, and a positive LE test.

tetanus- a disease marked by painful tonic muscular contractions caused by the neurotrophic toxin of *Clostridium tetani* acting upon the central nervous system.

titer- the standard of strength of volumetric test solution; the assay value of an unknown measure by volumetric means.

tolbutamides- an orally active hypoglycemic agent used in the management of adult-onset diabetes mellitus; it appears to stimulate the synthesis and the release of endogenous insulin from functional islets.

toxemia of pregnancy- an ill-defined term referring to metabolic disorders of pregnancy characterized by hypertension, edema, and albuminuria.

urobilinogen- precursor of urobilin.

zymogen- proenzyme; to trigger the release of an enzyme.

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